Effects of altering dietary roughage and concentrate proportions on fermentation and performance in beef cattle.

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Dedicated to Nathan Zeltwanger.

He gathers the lambs in his arms and carries them close to his heart; he gently leads those that have young. Isaiah 40:11

Overview

Alterations of beef cattle diets can lead to changes in both rumen fermentation and growth of animals. Proportions of roughage and concentrate in the diet have long been the focus of nutritionists and researchers as key components in altering rumen function, post-ruminal flow of nutrients, animal performance, and carcass characteristics. Two experiments were conducted to evaluate effects of varying dietary roughage and concentrate concentrations on feedlot performance, carcass characteristics, and rumen fermentation variables. Experiment 1 investigated effects of feeding a moderate-energy diet, as a result of increased roughage, on feedlot performance and carcass characteristics of steers receiving a two-stage, terminal implant. Steers received either a moderate-energy diet or a high-energy diet for 63 d prior to finishing to coincide with initial release of a growth-promoting implant. Growing phase dry matter intake (DMI) were greater ($P < 0.05$) for cattle consuming the moderate-energy ration, and this trend continued during the finishing phase ($P = 0.09$) and resulted in higher dry matter intake (DMI) over the duration of the experiment. However, no differences ($P > 0.05$) in average daily gain (ADG), feed conversion efficiency, final weight, and days on feed were detected at any point during the feeding period. Marbling score was decreased ($P < 0.05$) by feeding a moderate-energy diet, this was further evident by an increase in number of carcasses from cattle consuming the moderate-energy ration that graded select ($P < 0.05$). Experiment 2 utilized dual-flow, continuous culture fermenters to evaluate three different dietary roughage and concentrate concentrations supplemented with corn or flax oil on fermenter pH, digestion, nutrient metabolism, and fermentation products. Fermenter pH was lowered as dietary concentrate increased ($P < 0.05$); dry matter and organic matter digestion also increased ($P < 0.05$) with greater proportions of concentrate. Factors associated with nitrogen metabolism were

decreased ($P < 0.05$) as dietary roughage decreased; this may have resulted from lower pH values as well as differences in substrate. Total amount of volatile fatty acids was increased ($P < 0.05$) as more concentrate was included in the diet. Biohydrogenation of polyunsaturated fatty acids (PUFA) was reduced as dietary concentrate increased. This was likely more a result of decreased pH rather than dietary concentrate concentration. From these experiments it is concluded that altering roughage and concentrate concentrations increased DMI without impacting feedlot performance. Given performance of cattle was similar, marbling and carcass quality may have been impacted as a result of substrate rather than metabolizable energy intake. Rumen digestion and fermentation were improved by increasing dietary concentrate, while fermenter pH was decreased, and nitrogen metabolism was suppressed. Enzymatic breakdown of PUFA was limited as roughage concentrations were lowered in the diet. These results provide further evidence that altering dietary roughage and concentrate proportions can subsequently impact final products available to consumers.

Table of Contents.

List of Tables

List of Figures

Review of Literature

Introduction

Ruminant animals possess the unique ability to utilize fibrous plant material, which represents one of the world's largest carbon source (Holecheck et al., 2004), as a result of their fermentative chamber that houses a vast microbiome. Microbes enzymatically break down feed particles consumed by animals yielding, among other metabolites, volatile fatty acids (VFA) that serve as the energy source for the animal. Prior to World War II, cattle were raised on pasture or native range (NASEM, 2016), consuming diets made predominantly of forages. As more acres were converted to farming row crops, cattle raised for beef production transitioned into confinement feeding (feedlot). As a result, diets became more complex, utilizing more cereal grains, which lead to greater efforts in beef production research (Elam and Preston, 2004). One of the most extensively researched topics since that time has been optimizing the roughage:concentrate (R:C) ratio based on dry matter intake (DMI) and ADG responses.

Within the two distinct segments in which beef cattle are reared: cow-calf, and feedlot. Cow-calf producers operate primarily on forage-based systems, offering supplements to improve utilization of low-quality forages allowing cattle to meet their nutrient requirements with minimal harvested inputs. Backgrounding operations (feeding lightweight, calves a higher forage diet or permitting them to graze between weaning and feeding a high-energy diet) serve as a bridge between the cow-calf segment and the feedlots, allowing for a more consistent supply of uniform calves (Klopfenstein et al., 1999). Additionally, these operations also utilize diets comprised predominately of forages resulting in an increased R:C ratio. Cattle from both cow-calf and backgrounding segments

ultimately arrive in feedlots at various weights. Here cattle are fed a diet formulated to maximize growth and efficiency (NASEM, 2016).

In backgrounding there is less of an emphasis placed on maximizing growth; instead, increased attention is placed on extending the growth curve by delaying deposition of adipose tissue and increasing skeletal and muscle tissue (Sainz et al., 1995). By restricting growth, a period of compensatory growth is observed when cattle are transitioned into the finishing phase in the feedlots (Carstens, 1995). To maximize growth and efficiency higher amounts of cereal grains are fed to cattle during finishing resulting in a dramatically reduced R:C ratio.

In recent years, there is increased consumer interest in alternatives to grain-fed beef. Grass-fed cattle is far from a new concept, prior to WWII the majority of cattle were finished primarily on native range or improved pastures (NASEM, 2016); regardless of feeding strategy, most cattle consume forages for nearly their entire life (Mathews and Johnson 2013). However, as consumers increase their desire to be more conscious about the nutrient content of beef products, particularly fat profiles (Xue et al., 2010), there has been a resurgence in consumption of grass-fed beef. United States Department of Agriculture (2008) standards for grass-fed meat products require that animals have solely consumed forages, except for milk prior to weaning, throughout their lives. In addition, carcasses from grass-fed cattle must obtain an acceptable degree of finish in an economically feasible time frame (NASEM, 2016). Some individuals believe meat products from grass-fed cattle possess a healthier fatty-acid profile than traditionally grain-fed contemporaries due to greater concentrations of omega-3 fatty acids. Diet during the finishing phase plays a critical role in the quality and fatty acid profile of beef products

(Bidner et al., 1986). Roughage based diets contain higher concentrations of polyunsaturated fatty acids (PUFA), which have human health benefits (Griel and Kris-Etherton, 2006), than diets containing high levels of cereal grains (Daley et al., 2010). However, interest in supplementing traditionally fed cattle with fat sources to improve the fatty-acid composition of beef could offer an alternative strategy to grass-feeding programs in regard to improving its fatty acid profile.

Feeding cattle in the United States is an ever-changing proposition impacted primarily by consumer demands and producers' ability to meet that demand in an economically efficient way. As a result, a greater understanding of how nutrition and management within each beef industry segment impact the other, and how strategic approaches carried out by each segment play a role in meeting consumer demands. When executed correctly, cattle that enter the finishing phase from a backgrounding system reach similar degree of marbling as those that entered the feedlot without a backgrounding phase while yielding greater carcass weights (Lancaster et al., 2014). This phenomenon is critical for cattle feeders to produce increasing production of quality meet while relying on fewer resources. Also, grass-fed meat products continue to gain popularity amongst consumers for their improved fatty-acid profile; however, this led to additional research evaluating the feasibility of matching the fatty-acid profile of grass-fed beef in conventionally grain-fed beef by utilizing various fat supplements.

Factors impacting dry-matter intake

Variances in DMI have long been recognized as a complex component of the cattle feeding industry (Armsby and Fries, 1911). Dry-matter intake continues to be an extensive research topic, and rightfully so; how much an animal eats determines nutrient supply.

Hicks et al., (1990) stated that "DMI is the basis on which nutrient requirements, gain, and profit all are calculated or predicted, DMI must be estimated accurately." However, accurately predicting DMI is a significant challenge for nutritionist and cattle feeders (Forbes, 2003), as many factors affect DMI such as breed type, body composition, environment, and diet. Differences in DMI between sexes (steer vs. heifer) can be explained by differences in maturity at a given body weight rather than simply assuming difference is result of being a steer or heifer (NRC, 1987; NASEM 2016), Fox et al. (1988) suggested a frame-equivalent weight be used to predict DMI more accurately. As cattle mature, adipose tissue accrual limits DMI (Fox et al., 1998).These authors proposed that DMI should be adjusted down, regardless of sex, by 2.7% for every percentage unit increase in body fat content.

The mechanism by which accumulation of adipose tissue regulates DMI is not well understood; one explanation for reduced DMI may be reduced abdominal space resulting from adipose tissue deposition around the gastrointestinal tract (Taylor, 1969). Forbes (1980) proposed two ways by which adipose may influence DMI. First, there is a limit to the rate at which adipose tissue synthesizes triglycerides and, as that limit is approached, less energy is directed to adipose. Receptors found in the hypothalamic areas are sensitive to energy supply signal an energy excess, which in turn depresses DMI. Second, as metabolite uptake by adipose tissue is decreased, reduced metabolite uptake becomes a negative feedback leading to depression of DMI.

Age and previous background affect DMI. Cattle that entered the feedlot at either 3 or 7 months of age (weaning time) had lower DMI than cattle that entered after a specified time on pasture (Gill et al., 1993). The authors explained that lower DMI of young cattle

were due to decreased gut capacity. These results were consistent with those of Hickok et al. (1992), who observed that cattle placed cattle in the feedlot at 228 d of age had lower DMI than those that were placed in the feedlot at 444 d of age. Hill et al. (1993) placed fall born-cattle or yearling cattle on feed in July or September and proceeded to feed finishing diets composed of either rolled corn or steam-flaked corn to evaluate effects of age, date on feed, and corn processing on feedlot performance. When pooled across dates on feed and corn processing, calves consumed 0.77 kg/d less than yearling cattle; yearling cattle were permitted to graze longer before being fed a finishing diet (Hill et al., 1993). While older cattle are assumed to accumulate more adipose, previous background can impact body composition (Sainz et al., 1995). Steers of similar age exposed to ether low energy diet or high energy diet (high roughage or high concentrate) resulted in differing body composition and similar carcass weights after the growing phase. Cattle consuming more roughage had greater DMI and increased GI tract weights, however, carcass fat was lower.

Hicks et al., (1990) reviewed A review of feed records from 2,051 pens (296,367 cattle) demonstrated that, at greater feedlot placement weights, cattle had greater average DMI. This relationship is not linear. Figure 1.1 (Hicks et al., 1990) illustrates that, soon after arrival in the feedlot, there is a sharp increase in DMI, regardless of placement weight. Dry-matter intake continues to increase gradually followed by a slight decrease as cattle mature. Figure 1.1 also demonstrates how three DMI-effecting factors, in-weight, age, and composition, influence DMI. The decrease in DMI as cattle mature (Figure 1.1) represents a regulation of DMI beyond gut fill as discussed by NRC (1987). Cattle entering the feedlot after an extended period of grazing or consuming high roughage diets will likely have greater intakes as a result of greater gut capacity and lower carcass fat. Sainz et al. (1995)

demonstrated that feeding a high roughage diet resulted in greater GI tract weights and lower carcass fat content than steers consuming a high energy diet.

Diet effects on DMI in feedlot cattle are direct through roughage concentration. Adding roughage to diets is accomplished by incorporating fibrous feedstuffs. Fibrous feedstuffs are commonly analyzed for acid detergent and neutral detergent fiber (ADF and NDF respectively), where NDF represents all structural carbohydrates, hemicellulose, cellulose, and lignin, and ADF represents cellulose and lignin. At weaning, beef calves proceed to one of two systems: a growing or finishing system (Peel, 2003). A growing system is defined by placing cattle on pasture (stocker), feeding a high-roughage diet (backgrounding), or limit-feeding a high-roughage or high-grain diet. Backgrounding systems are commonly used throughout the Midwest, which utilize fermented fibrous feedstuffs, such as corn silage at > 50% of diet DM basis leading to greater dietary ADF and NDF concentrations (NASEM, 2016). When cattle are placed on a backgrounding diet, DMI generally exceeds DMI by cattle of similar weight and body composition fed a high-energy ration (6% to 10% of diet DM as roughage; Samuelson et al., 2016). Gill et al., (1976) fed diets (DM basis) containing 14%, 30% or 75% corn silage with and without monensin, to 278-kg steers. When not supplemented with monensin, DMI was 7.74, 8.53, and 8.41 for steers consuming 14%, 30%, or 75% corn silage, respectively. These results agree with Gill et al., (1976) demonstrating that even though increased roughage will in turn increase DMI, gut fill can be a limiting factor in high roughage diets in diets that are primarily roughage. When feeding 227 kg weanling steers either low or high energy diets, Ralston et al., (1966) observed DMI were lower for steers consuming pellets containing higher levels of concentrate.

Galyean and Defoor (2003) reviewed data from 11 manuscripts and compared 48 treatment means to evaluate effects of roughage source and concentration on DMI by feedlot cattle. Surveys on feedlot consultant recommendations including those for roughage concentration in feedlot diets were provided previously by Galyean and Gleghorn (2001), Vasconcelos and Galyean (2007), and Samuelson et al., (2016). These authors concluded that feedlot consulting nutritionist formulate diets to contain (DM basis) from 4.5% to 13.5%, from 4.5% to 13.5% or from 6.0% to 12.0% roughage, respectively.

Adding low concentrations of roughage to high-energy diets serves two primary roles: to help prevent digestive upsets and to maximize energy DMI (Galyean and Defoor., 2003). Roughage concentration and source influences DMI as well; dietary roughage concentration accounted for 69.1% of DMI variability, however roughage NDF concentration accounted for a greater portion of the variation (92.0%) in DMI (Galyean and Defoor., 2003). These results demonstrate that when formulating finishing diets, NDF from roughage should be utilized to predict DMI. Lambs fed diets containing 55% or 72.5% or consumed more DMI than those fed 90% concentrate pellets (Hudson et al., 1989). While there were no DMI differences for lambs consuming 55% or 72.5% pellets, feed conversion efficiency was greater for lambs fed 72.5% or 90% concentrate pellets. In that study, intake of net energy for gain (NE_g) was similar across dietary concentrate treatments, which in turn led to no differences in ADG. Authors of that study attributed poor feed conversion of lambs fed 55% concentrate pellet to the low energy content of that diet.

Increasing roughage content from 0% to 15% in diets fed to 334-kg steers linearly increased DMI (Kreikemeier et al., 1990). Average daily gain response was quadratic:

greatest ADG were reported by cattle consuming 10% roughage diet. Consequently, feed:gain was lowest for cattle consuming diets containing 5% or 10% roughage. The authors concluded that while cattle fed the 15% roughage diet consumed more feed, greater DMI did not lead to greater ADG due to dilution of energy resulting from an increase in dietary roughage concentration. Interestingly, cattle consuming diets containing 0% roughage consumed the least amount of feed, had the poorest ADG and feed conversion efficiency demonstrating the importance of dietary roughage. Defoor et al., (2002) evaluated effects of roughage concentration and source in 275-kg heifers. In one experiment cottonseed hulls (CSH) and sorghum Sudan silage (SSIL) were included (DM basis) in the diet at 2.5%, 5.9% or 12.5% and 3.2%, 7.1% or 12.5%, respectively; a negative control diet containing 12.5 % alfalfa hay (ALF) was also included. At 12.5% inclusion, roughage sources were compared on an equal dietary inclusion basis. When diets contained 5.9% CSH, 7.1% SSIL or 12.5% ALF, diets contained similar NDF concentration. Lastly, CSH and SSIL were mixed into the diet at 2.5 and 3.2% to provide equal NDF from particles >2.36 mm (retained NDF) as 12.5% ALF. When roughage sources were fed at similar DM inclusion, gain, and DMI were greatest for cattle fed CSH, intermediate for those fed SSIL, and lowest for cattle fed ALF. The authors concluded that roughage value, effectiveness of roughage for supporting NE_g DMI, for CSH and SSIL be higher than ALF. When roughage sources were compared at similar contribution to dietary NDF, DMI was similar for cattle fed CSH and ALF, but those fed 7.1% SSIL consumed more DM than those fed 12.5% ALF. At the lowest dietary NDF concentration (2.5% CSH or 3.2% SSIL) cattle fed CSH consumed less but those fed SSIL consumer more feed than cattle fed 12.5% ALF. These results indicate that SSIL and ALF may need to be exchanged on a retained

NDF rate rather than similar percentage of NDF as demonstrated by similar DMI for 5.9% CSH and 12.5% ALF.

Diet effects on passage rate

Passage rates (PR) of ingested feed are impacted by roughage concentration and level of DMI, consequently site of digestion and nutrient absorption can be impacted also. Rumen contents are generally classified into two separate phases, liquid and solid, and each phase is subdivided into three respective pools; namely, A, B, and C (Owens and Goetsch, 1986). Rumen fluid that is free and not associated with any particles is designated as pool A. Pool B is defined as fluid imbedded in particles of the correct size and density to pass from the rumen in their current state, while fluid from pool C is associated with particles that are too large to pass from the rumen in their current state. Fluid passage rate (FPR) is increased after feeding (Teeter and Owens 1983). Volume of pool A is likely highest prior to feeding as the concentration of feed particles is low (Owens and Goetsch 1986). Following a meal, an influx of water would enter pool A but quickly moved into pools B and C as feed particles become saturated with liquid increasing the likelihood of passage from the rumen (Ehle 1984). Fluid passage rate is higher for diets containing high roughage concentrations (Warner 1981). Cole et al., (1976) explained that this is likely the result of increased mastication and saliva secretion. Additionally, a portion of ingested roughage should become saturated with free liquid from pool A and lead to increase passage from the rumen. As roughage increases in a high concentrate diet, more significant amounts of pool A and B will pass through the rumen (Owens and Goetsch 1986). Mudgal et al., (1982) demonstrated that FPR increased when adding roughage to high-grain diets.

Solid passage rate (SPR) was also broken divided into three pools A, B, and C by Owens and Goetsch (1986). Particle pool A includes soluble nutrients and small feed particles which flow with fluid pool A. Particles found in pool B would primarily come from high concentrate rations, and coarse roughages predominantly make up particle pool C. With increased rumination and mastication, particles from pool C should be moved to pools B and A after particle size reduction, which would allow passage from the rumen. Indigestible particles need to be processed before feeding for them to pass through the rumen. Grinding and pelleting less digestible feedstuffs has been shown to avoid selective retention in the rumen (Van Soest 1982). Evans (1981) observed that solid passage rate is positively related with roughage level: SPR was lower when diets contained > 80% concentrate.

Increasing roughage in the diet, as addition of indigestible material, increased ruminal outflow of concentrate particles from the rumen (Wheeler et al., 1979). However, increasing DMI may not have the same effect on SPR as it was reported to have on FPR. Galyean and Chabot (1981) reported that as DMI increased, compensatory increases in rumen volume offset DMI changes resulting in similar SPR. Dry-matter intake of high concentrate diets can, however, influence FPR and alter site of digestion (Owens and Goetsch, 1986). Overall, Mäkelä (1956) concluded that roughage intake had a more significant effect than concentrate intake on SPR. Additionally, if roughage is added to high concentrate diets rumen gas production increased altering specific gravity of the rumen forcing smaller particles trapped in the rumen mat to sink and pass from the rumen (Owens and Goetsch, 1988) Roughage level and DMI are two factors commonly associated with PR and altering site of digestion and absorption of nutrients to the lower GI tract

(Varga, 1995). However, given limited absorption capacity of the small intestines increasing PR can lead to a decrease in total tract digestibility of high roughage diets (Galyean and Ownes, 1989).

Diet effects on rumen pH and buffering

Addition of concentrate to ruminant diets generally results in lowering ruminal pH. In forage-fed ruminants, rumen pH ranges from 5.8 to 6.8. In contrast, it is not uncommon for pH to drop below this range when cattle are fed high concentrate diets (Fuentez et al., 2009).

Lowering ruminal pH results in various effects in the rumen and animal. Nagaraja and Titgemeyer (2007) indicated that, as ruminal pH was lowered, the extent and rate of fiber digestion also was reduced. At ruminal pH less than 5.5, Owens et al., (1998) reported signs of subclinical acidosis. Nagaraja and Titgemeyer (2007) suggested a drop in rumen pH below 5.0 to signify acute acidosis; similarly, ruminal lactate concentration > 50 mM also represents acute acidosis. As dietary R:C decreases, rapidly fermentable carbohydrates, mainly starch from cereal grain, are digested in the rumen leading to a more acidic environment as a result of increased VFA production. Volatile fatty acids are removed from the rumen by either absorption or passage (Penner et al., 2009); at a lower pH, absorption is reduced as a result of damaged rumen epithelia, and passage rate is reduced as a result of lower DMI and rumen motility (Fulton et al., 1979; Wilson et al., 2012). These effects result in lower rate of VFA removal leading to VFA, which further decreases rumen pH (Penner et al., 2009).

Saliva serves as an essential buffer for the rumen because it is estimated to neutralize 40% of acid produced in the rumen (Allen, 1997). In addition, saliva is a vehicle

for recycling nitrogen and contributes mucin proteins that function to prevent bloat (NASEM, 2016). Saliva contains sodium, potassium, bicarbonate, phosphate, and other inorganic compounds (Bailey and Balch, 1961), and a pH of about 8.2 (McDougall, 1948). Bicarbonate supplied by saliva (Bailey and Balch, 1961) or rumen epithelia (Penner et al., 2009) serves as the primary neutralizer of hydrogen ions. With a 6.1 pKa value similar to rumen pH (Aschenbach et al., 2011), bicarbonate reacts with hydrogen ions producing water and CO_2 (Allen, 1997), which aids in buffering low rumen pH. Hydrogen phosphate serves as a weak base rather than a true buffer, given its pKa value of 7.2 (Kohn and Dunlap, 1998) it acts to neutralize protons at any pH and remove them through with passage to the omasum (NASEM, 2016). Elevated roughage levels aids in buffering rumen pH by forming a rumen mat that is suspended in the rumen (Owens and Goetsch, 1986), leading to an increase in rumination, and thereby, an increase in saliva secretion. Roughage NDF is also more slowly digested than starch, decreasing overall rate of carbohydrate fermentation and ultimately rate of VFA production (NASEM, 2016). Differences in rumen pH will exist due to varying concentrate levels in diets, regardless of buffering capabilities (Fuentes et al., 2009). Consequently, rumen digestion and metabolism will also be impacted as enzyme activity is often pH sensitive (Bach et al., 2005; Jenkins et al., 2008).

Rumen ecology

The distinguishing feature of the ruminant digestive system is a four-compartment stomach: reticulum, rumen, omasum, and abomasum. The abomasum most closely resembles the stomach of a monogastric animal, with the reticulum, rumen, and omasum comprising the forestomach and serving as the site for anaerobic fermentation. The rumen is a large anaerobic, fermentative chamber that possesses a highly diversified microbial

population capable of digesting feed particles that would be unusable by other species. Occasionally, oxygen is presented to the rumen with ingested feed where it is quickly consumed by facultative anaerobic bacteria (NASEM, 2016). The rumen environment is tightly regulated at a temperature of 39 ºC and a normal pH range between 5.8 and 6.8 for animals consuming primarily forage diets. Three distinct groups of microorganisms inhabit the rumen, consisting of bacteria, protozoa, and fungi (Russell, 2002). By utilizing a variety specialized enzymes, rumen microbes can convert feed particles to end-products of fermentation. The main products of rumen fermentation are volatile fatty acids (VFA) acetate, propionate, and butyrate. Volatile fatty acids are absorbed mainly across the rumen wall and contribute mainly to meeting each animal's energy requirements. Additionally, ammonia, CO_2, CH_4, and H_2 are also generated during ruminal fermentation.

Bacteria

Rumen bacteria account for the largest portion of the ruminal microbial population (Russell, 2002), and their growth/efficiency varies considerably with rumen pH, one of the most variable ecological factors (Yokoyama and Johnson, 1988). More than 50 genera of bacteria have been identified within the rumen (Russell, 2002), and their functions vary substantially based on substrate. Cellulolytic bacteria are responsible for degrading cellulose and partially hemicellulose (Yokoyama and Johnson, 1988). Amylolytic bacteria are more prominent when high-concentrate diets are fed, and play a critical role in the digestion of starch, as well as, simple sugars (Russell, 2002). Additionally, amylolytic bacteria degrade cellulose when high concentrate diets are fed, however, with diets high in roughage amylolytic bacteria degrade protein (Wallace et al., 1997). This demonstrates that type of substrate determines nitrogen metabolism (Bach and Stern, 2005), and that of

other nutrients. Lypolytic bacteria work to break down fat by hydrolyzing ester bonds to separate fatty acids (FA) from their glycerol backbone (NASEM, 2016). Proteolytic bacteria are responsible for the degradation of protein.

Protozoa

Ciliated protozoa are substantially larger in size than bacteria (William and Coleman, 1997), and account for up to 50% of rumen biomass (Nolan, 1993; Russel, 2002). Protozoa can engulf and degrade starch and sugar molecules (Williams and Coleman, 1997). Additionally, protozoa prey on bacteria to derive their N source (Bach and Stern, 2005). Protozoa are not critical for rumen function. Jouany et al., (1988) defaunated the rumen and demonstrated that metabolism of various nutrients continued in the absence of protozoa. Further, Karma (2005) reported that feed conversion efficiency was improved, particularly in high forage diets.

Fungi

The role of anaerobic fungi in ruminant metabolism is not as well understood as that of bacteria and protozoa; however, there is strong evidence that they play a critical role in fiber digestion, particularly low-quality forages (NASEM, 2016). Vargra and Klover (1997) demonstrated that fungi were able to penetrate cell walls and degrade cellulose from within the plant. Additionally, Kamra (2005) reduced gas production in vitro when fungi were removed from rumen inoculum. High concentrate diets inhibit the growth of rumen fungi, Orpin (1994) stated this is likely due to a reduction in rumen pH.

Carbohydrate fermentation

Carbohydrate metabolism and formation of VFA in the rumen supply 60% to 75% of digestible energy (Sutton, 1979). Rumen microbes utilize cellulose, hemicellulose, pectin, starch, and sugars, and up to 90% of carbohydrates are digested in the rumen (NASEM, 2016). Carbohydrates are hydrolytically digested and produced a number of end-products, including the VFA: acetate, propionate, and butyrate. Before VFA are formed, carbohydrates are degraded primarily to hexoses and converted to pyruvate by microorganisms mostly through the glycolysis pathway (Russell and Wallace, 1988). Substrate fermentation determines VFA yield and concentrations. Feeding high forage diets produce acetate, propionate and butyrate in a 70:20:10 proportion (Owens and Goetsch, 1988). When high concentrate diets are fed to ruminants, proportions of these VFA are changed to 50:40:10 (Bevans et al., 2005). Additionally, total ruminal VFA concentration is also affected by diet. France and Siddons (1993) demonstrated that normal VFA concentrations in ruminants fed 100% to < 40% roughage is 70 to 130 mM, but these values are known to range from 30 to 200 mM based on diet composition and DMI.

The critical distinction between the three main VFA produced in the rumen are the number of carbons, and subsequent utilization. Acetate, a 2-carbon compound, is most prominent in high-forage diets. Following absorption from the rumen, acetate is oxidized for energy in the Krebs cycle or used as substrate for de novo fat synthesis (Hood et al., 1972). Acetyl-CoA synthetase is highly active in extrahepatic tissue (Cook et al., 1968). Acetyl-CoA activity is 2 to 3 times greater in adipose tissue, reflecting higher affinity of acetate to adipose tissues. High concentrate diets generate higher concentrations of propionate, a 3-carbon compound. Under normal conditions, nearly all propionate is removed from the blood by the liver (Cook and Miller, 1965). Propionate is critical for

glucose production as it is the only VFA capable of providing carbon for gluconeogenesis (NASEM, 2016). Lindsay (1970) reported that 27% to 54% of glucose is synthesized from propionate; the balance of glucose synthesized by ruminants is derived from gluconeogenic amino acids (Brockman, 2005). An estimate of 2 to 5% of glucose is converted to lactate and stays in the rumen (Fahey and Berger, 1988). In times of increased propionate concentrations in circulation, several tissues including adipose can metabolize propionate (Cook et al., 1968). Little is understood about utilization of the 4-carbon compound, butyrate; but Li et al., (2012) stated that it is critical for rumen health and papilla growth. Butyryl-CoA synthetase is responsible for converting butyrate to β-hydroxyl butyrate (Cook et al., 1968) to prevent hyperglycemia when transported in blood (Phillips and Black, 1966). Ultimately β-hydroxyl butyrate can be converted to ketone bodies to be utilized for fatty acid synthesis in adipose tissue or oxidized in skeletal and cardiac muscle (Cook et al., 1968).

Lipid metabolism

Increasing energy content of finishing diets through fat inclusion led producers to utilizing fat supplements (M. De Beni Arrigoni et al., 2016). Adding fat to finishing diets improves ADG and overall feed conversion efficiency (Zinn, 1989; Krehbiel et al., 1995). However, fat may lead to lower fiber digestibility (Zinn, 1989) possibly offsetting the potential energy contribution (Krehbiel et al., 1995).On the other hand, Daley et al. (2010) indicated that consumers are becoming more interested in health factors associated with consumption of beef products. Therefore, supplementing beef cattle diets with certain fat sources may play a role in altering fatty acid composition of beef products. Although most plant-based feedstuffs used in beef cattle diets contain low lipid concentration,

supplemental energy sources such as oilseeds have substantially greater lipid concentrations (Palmquist, 1988). Lipids in forages are concentrated mainly in the leaf of each plant, and fatty acids (FA) are comprised primarily of glycolipids and phospholipids (VanSoest, 1994). Alternatively, triglycerides are predominately found in concentrate feedstuffs, such as cereal grains (VanSoest, 1994) and energy supplements. Forages contain greater amounts of unsaturated fatty acids including oleic, linoleic, and α-linolenic (18:1, 18:2, and 18:3, respectively) acids (Palmquist, 1988). Even though forages are higher in polyunsaturated fatty acids (PUFA) concentration, simply increasing their concentration in the diet does lead to greater content of PUFA in meat (NASEM, 2016). Unsaturated fatty acids are toxic to certain strains of rumen bacteria. As a measure of protection against toxicity, biohydrogenation is hypothesized to occur to reduce toxic effects of PUFA (Margarida et al., 2006). Before biohydrogenation can commence, lipolysis of the ester bonds in a triglyceride must take place (Jenkins, 1993). Following lipolysis, isomerization from cis- to trans- formation is then followed by hydrogenation of double bonds (Jenkins et al., 2008; Figure 1.2). Two primary PUFA substrates for biohydrogenation are C18:3, found primarily in forage, and C18:2 mainly from cereal grains. During biohydrogenation of linoleic acid, various conjugated linoleic acids (CLA) are formed: cis-9, trans-11-18:2 is one of the most common (Jenkins et al., 2008). Incomplete biohydrogenation of α-linolenic resulted in greater concentrations of trans-11-18:1 (Maia et al., 2006). Polen et al. (1964) demonstrated that *Butyrivibrio fibrisolvens* is involved in biohydrogenation. Maia et al. (2006) identified three strains of *Butyrivibrio* and two strains of *Clostridium* participating in biohydrogenation. *Clostridium* was identified as the only bacteria that produced stearic acid (C18:0) from biohydrogenation.

Protozoa do not contribute to the process of biohydrogenation. Biohydrogenation activity decreased only slightly in a defauntated rumen (Dawson and Kemp, 1969). Higher concentrations of USFA protozoa are thought to be the result of ingesting bacteria. (Singh and Hawkel, 1979; Hartfoot and Hazelwood, 1997). Others reported that USFA found in protozoa result from engulfing plant components (Stern et al., 1977). However, this did not explain the elevated trans-11-18:1 concentrations as this FA is not a component of plant lipids, (Jenkins et al., 2008). Protozoa flow from the rumen makes FA available to the lower gut (Jenkins et al., 2008). Through this process, vaccenic acid becomes substrate for de novo synthesis of cis-9, trans-11-18:2; a CLA known to have human health benefits (Daley, 2010).

Fungi contain high concentrations of oleic FA (Kemp, 1984). Maia et al. (2006) grew strains of fungi in a linoleic acid compound and after 96 h only half of the compound had been utilized. This rate of utilization is small compared to that observed by *B. fibrisolvens*; their rate of linoleic acid utilization takes only a few minutes (Maia et al., 2006).

Recently, decreasing biohydrogenation activity was proposed as a way to increase flow of PUFA, particularly n-3 fatty acids post-ruminally. Ruminal pH manipulation presents an opportunity to modulate biohydrogenation. Effects of changes in pH on biohydrogenation were studied (Van Nevel and Demeyer 1995; Jenkins et al., 2009). Van Nevel and Demeyer (1995) collect rumen fluid from sheep that were fed either a commercial concentrate or meadow hay. Rumen fluid was then placed in a glass flask, incubated with buffer solution and a nitrogen source; each sample was then adjusted to a specific pH with addition of HCl. These authors reported that pH had a greater impact than

substrate on altering biohydrogenation, and that lipolysis, the first step of biohydrogenation, was inhibited more than hydrogenation. Jenkins et al. (2009) observed similar results in dual-flow continuous culture fermenters. Fermenters were inoculated with rumen fluid and fed either a low- or high-concentrate diet. Fermenter pH was maintained at either 6.4 or 5.6 with the addition of acid or base to investigate the effects of either substrate or pH on biohydrogenation. Again, pH had a greater impact than substrate on lipid transformations in vitro, and lipolysis was impacted more than hydrogenation. Inhibiting lipolysis prevents hydrogenation and potentially provides a way to increase flow of n-3 fatty acids from the rumen.

Importance of α-linolenic acid

Alpha-linolenic acid (cis-9, cis-11-cis-15-18:3; ALA) is considered an essential fatty acid because it cannot be synthesized by either ruminants or humans. Increasing flow of ALA, an omega-3 (n-3), increased concentrations of ALA and other PUFA, deemed to possess certain human health benefits, to be deposited in the meat (Cherfaoui et al., 2011). Doctors recommend that consumers increase their intake of ALA to help reduce the omega-6 to omega-3 ration from 15:1 to 20:1 (Simpopoulos, 2006) to the recommended ratio of 4:1 (Daley, 2010). Increasing ALA concentration in meat is directly related to the diet fed to cattle (Wood et al., 2004). Fresh grass is comprised of 50% to 75% ALA, whereas, grain-based diets are comprised of linoleic fatty acids, an omega-6 FA (Hawke, 1973). Nuernberg et al. (2005) reported that omega-6 to omega-3 ratios were reduced from 7:1 to 2:1 when cattle were fed grass. Comparisons derived from grain-fed and grass-fed cattle were reviewed by Daley et al. (2010). Meat from grain-fed cattle had greater total lipid

concentrations. Concentrations of alpha-linolenic acid and vaccenic acid in meat of grass-fed cattle were consistently greater than those in meat of grain-fed cattle.

Jenkins et al. (2008) stated that interest to supplement fat was growing not only to increase energy density of the diet, but also a mechanism to improve concentrations of ALA and other fatty acids thought to have health benefits. Tallow and yellow grease are the most commonly used sources of fat in beef cattle diets (NASEM, 2016). Feeding vegetable or fish-based oils result in greater yield of biohydrogenation intermediates, increasing their availability for metabolism by various tissues (Bauman et al., 2011). Compared to vegetable oils, feeding animal fats increased the ratio of SFA to USFA (Hess et al., 2008). Feeding holstein bulls diets enriched with ALA increased ALA and other PUFA content in meat (Herdmann et al., 2010). Given the toxicity of PUFA on rumen microbes and effectiveness of biohydrogenation, protection of PUFA in the rumen is critical to increase their availability to be deposited in meat or milk. Currently, there are two primary approaches to protecting PUFA from biohydrogenation: altering the structure of FA to resist actions of microbial enzymes and encapsulating USFA (Jenkins and Bridges, 2007). When analyzed as a percentage of intake, ruminal hydrogenation of USFA were similar for protected and unprotected fats (Jenkins and Bridges, 2007).

Rumen function in vitro

Studying rumen function in vitro provides an alternative to costly in vivo studies (Stern et al., 1997). Other benefits of studying rumen function in vitro include reduction of the impact of animal variation and a system to test effects of toxic compounds (Hristov et al., 2012).

Continuous culture (CC) rumen fermenters were developed to model rumen fermentation in vitro. These systems are unique because they permit removal of fermentation end-products thereby permitting a fermentation to be maintained for a longer duration of time. Early systems developed by Alder et al. (1958) could only maintain steady-state fermentation for up to 10 h. Stewart et al., (1961) was able to increase fermentation time to 24 h by constantly adding substrate and removing effluent. In the system developed by Slayter et al. (1967) steady-state fermentation was maintained for up to 7 d. Further, observations on rumen fermentation resulting from this system were similar to those made in vivo.

A critical problem that persisted through early development of CC was their inability to maintain protozoa population size similar to those observed in vivo. Through differential solid and liquid flow rates of fermenter effluent Hoover et al. (1976) developed a CC system that permitted protozoa populations larger than those reported earlier (Slyter et al., 1967). In spite of this improvement, protozoa populations were smaller than those reported in literature for in vivo studies (Hoover et al., 1976).

Hannah et al. (1986) modified the system outlined by Hoover et al. (1976) by decreasing fermenter volume, utilizing N_2 gas to create an anaerobic environment, and added a coaxial heating element to maintain inoculant temperature similar to in vivo ruminal temperature. Hannah et al. (1986) fed identical diets to either cannulated dairy cows or dual-flow continuous culture fermenters to compare difference fermentation parameters. Organic matter, crude protein and amino acid degradability values derived by system developed by Hannah et al. (1986) were similar to those found in vivo from cows fed identical diets. When reported as mol/100 mol acetate: propionate ratios were similar

to those reported in vivo. Concentrations of VFA reported for CC systems are greater than those reported from in vivo studies. Lack of VFA absorption in CC systems is cited as the reason for this difference. Ultimately, Hannah et al. (1986) determined their system provide reasonable estimation for rumen fermentation when compared with *in vivo* results from dairy cows fed identical diets. Mansfield et al. (1995) conducted a similar experiment utilizing a similar system outlined by Hannah et al. (1986) modified to control fermenter pH by addition of either a weak acid or base. Similar diets were fed to both dairy cows and fermenter to investigate culture method and its effect on fermentation. Concentrations of VFA and total bacteria populations were greater in CC than for *in vivo* rumen fermentation. Fewer total bacteria were attributed to less competition over substrate in the absence of protozoa (Mansfield et al., 1995). Amylolytic and proteolytic bacteria concentrations were similar between in vitro and in vivo systems, but cellulolytic bacteria were greater in the CC system. Digestion of OM *in vitro* did not differ from *in vivo* values. Crude protein and NDF digestion were, however, impacted by culture method; NDF digestion was higher *in vivo* than CC (58.3% vs 47.4% respectively), CP digestion however, was greater in CC (42.9% vs 38.8%). Overall, Mansfield et al. (1995) estimated that results from their CC system were similar for 80% of individual parameters measured when compared to in vivo results from similar diets.

Factors affecting growth and development

Limits to animal growth and body composition are set by genetics. Through enhanced technologies, and our understanding of their capabilities, the aptitude for an animal to reach or express that potential can be impacted. Composition, rate, and length of growth can all be impacted by an animal's previous and current plane of nutrition, and with

the addition of technologies, such as implants and various feed additives producers can structure their management to adequately meet endpoints they deemed profitable.

Backgrounding

Altering roughage content changes the energy density of a diet (Defoor and Galyean 2002). Backgrounding diets traditionally feed greater amounts of roughage, altering the R:C ratio, impacting an animal's growth and development. There are three primary purposes for backgrounding operations: firstly, backgrounding offers a marketing channel for roughage sources (Sip and Pritchard, 1991). Secondly, backgrounding allows for an increase in carcass size without excessive fat deposition, particularly in smaller cattle (Byers, 1982). Lastly, Klopfenstein et al. (1999) state that backgrounding operations act as a bridge between cow-calf producers and the feedlot, allowing a more consistent flow of animals entering the feedlot throughout the year. Meyers et al., (1965) demonstrated that feeding a high energy diet ad libitum led to greater carcass fat deposition, while Berg and Butterfield (1968) reported fat deposition was retarded when a low energy diet was fed to cattle. During backgrounding, carcass fat mass varies with energy intake and is lower for calves that were energy restricted for long periods of time (Owens et al., 1993). Sainz et al., (1995) evaluated carcass composition of cattle fed either a high concentrate diet ad libitum, a high concentrate diet fed at restricted DMI, or a forage-based diet fed ad libitum. Carcass and empty-body fat were greatest for cattle given ad libitum access to the high concentrate diet. Empty-body protein was highest for cattle restricted on energy (Sainz et al., 1995). Even though fat deposition is reduced during the growing phase when a high roughage-based diet, this effect is not irreversible. Lawrence and Pearce, (1964) showed that a low level of nutrition did not impact carcass composition when given adequate

periods of unrestricted recovery. This is consistent with more recent research when cattle are adjusted or finished to a constant fat endpoint (Klopfenstein et al., 1999; Loken et al., 2009). Reducing ADG also has a similar impact of growth and development of ruminants. Byers (1980) reported that as backgrounding ADG increased both fat and protein accretion increased, however, fat appeared to increase at an increasing rate while protein seemed to increase at a decreasing rate. Owens et al. (1995) concluded the amount of fat accretion during backgrounding is dependent on energy intake, and research by Gill et al. (1993) found calves grazing 122 d during summer were less fat than calves that grazed for only 68 d. Unlike fat, protein accretion does not appear to plateau suggesting that cattle capable of increased ADG may deposit more protein per unit of fat during backgrounding. Ultimately, Owens et al. (1995) concluded that fat accretion in rapid gaining cattle tend to reach a plateau that could limit both energy and DMI.

Backgrounding effects on feedlot and carcass performance

While increasing ADG during backgrounding is not the purpose of backgrounding (Peel, 2003) it can impact performance during finishing when compared to cattle fed a high-energy diet during the growing phase (McCurdy et al., 2010). Drouillard et al. (1990) fed 0.66 or 0.75 net-energy for gain (NE_g) diets for 77 d and compared them against cattle fed ad libitum either 1.26 NE_g growing diet or 1.61 NE_g finishing diet. Finishing phase ADG was mostly higher for cattle initially fed a lower energy diet. However, when cattle fed were fed for either 1.06 NE_g or 1.19 NE_g ADG during the growing phase, Loken et al. (2009) reported no difference in finishing phase performance. These observations differed from those of Neel et al. (2007) who fed cattle to achieve low, medium, or high ADG (0.23, 0.45, or 0.68 kg/d respectively) during backgrounding. Cattle fed to gain to gain 0.23 kg/d

during backgrounding had the greatest ADG during finishing. Additionally, when Coleman et al. (1995) fed either a silage-based (0.62 NE_g) or grain-based (1.21 NE_g) diet during backgrounding, finishing phase ADG was highest for cattle fed the silage based diet. In the study by Loken et al. (2009) whole shelled corn was used as a primary energy source during finishing. Whole corn contains lower NE_g than corn that has been processed (Zinn et al., 2002). Feeding whole shelled corn during the finishing phase may have restricted energy and ADG, and may explain why cattle fed by Loken et al. (2009) had similar ADG during finishing regardless of background treatment.

Feeding higher amounts of roughage in a diet, as it occurs during backgrounding, generally results in greater DMI both during the growing and finishing phase (Peel, 2003). Even though ADG is mainly higher during finishing, greater DMI usually result in overall efficiencies to be similar or favorable for cattle fed high concentrate diets. Drouillard et al. (1990) discovered feed efficiency was similar for control cattle fed a finishing control diet compared with a growing control diet and three different levels of energy restriction, similar results were reported by Loken et al. (2009) who found gain:feed to be similar for either 1.06 NE_g or 1.19 NE_g diets during backgrounding. These results were later replicated by McCurdy et al. (2010) who reported similar gain: feed observations for cattle that were fed either silage based diet or programed fed designed to reduce ADG with cattle given ad libitum access to a high concentrate diet. However, Sainz et al. (1995) reported empty-body weight gain:feed to be highest during finishing for cattle experiencing a form of energy restriction during the growing phase when followed by ad libitum access to a high concentrate diet. Cattle limit fed a high energy diet prior to finishing had similar efficiency as cattle with unrestricted energy intake. When energy intake during growing phase was

lower, as a result of forage inclusion, overall efficiency was lowest, these results can be explained by increased DMI and lower ADG throughout the study.

Two primary backgrounding systems exist with variation within each system (Peel, 2003). First, cattle can be backgrounded on a grazing-based system where cattle graze a variety of forages such as wheat pasture, native range, improved pasture, cover crops, or crop residues (McCurdy et al., 2010; Kumar et al., 2012; Cox-O'Neil et al., 2017). The primary role of these backgrounding systems is to utilize forages that only ruminants can (Sip and Pritchard, 1991). The second backgrounding system occurs in a drylot where a various diet may be fed to limit energy consumption. Commonly silage-based diets are fed to reduce the energy value of the ration, preventing excess energy intake, and reducing fat deposition during the growing phase.

Additionally, producers can restrict energy intake by physically restricting DMI of a high concentrate diet (Owens et al., 1993; Sainz et al., 1995). This purpose of this method is to prevent excessive development of adipose during the growing phase. McCurdy et al. (2010) investigated the impacts of different growing programs: grazing wheat pasture, feeding a silage-based diet, or program fed a high-concentrate diet, and ab libitum fed a high concentrate diet. Finishing phase ADG was found to be highest for silage-fed cattle, intermediate for program-fed cattle, and lowest for wheat pasture cattle. Gain: feed followed a similar trend with silage-fed and program-fed cattle being more efficient than cattle grazing wheat pasture. Kumar et al. (2012) compared three backgrounding treatments that consisted of grazing either barley or millet and dry lot treatment in which cattle were given ad libitum access to grass-legume hay. During finishing, ADG and gain:feed were similar across all treatments. Cox-O'Neil (2017) conducted a two-year experiment with

three background treatments consisting of grazing corn residue plus a dried distiller's grain supplement, grazing an oat-brassica forage mix, or feeding a silage-based diet in a drylot setting. Performance varied significantly by year and by treatment. In year one, ADG was relatively similar across treatments with gain:feed being highest for cattle in the drylot. In year two, ADG was highest for cattle grazing corn residue, lowest for cattle consuming corn silage, and intermediate for animals grazing cover crops. This experiment demonstrates the impact weather can have on different backgrounding systems (Cox-O'Neil et al., 2017). Sainz et al. (1995) initially limit fed a high concentrate diet or ad libitum fed a high roughage diet prior to finishing and compared results with cattle fed ad libitum a high concentrate diet for the duration of the study. Finishing DMI was lowest for cattle consuming the high concentrate diet ad libitum. Daily empty body weight gain during finishing was higher when cattle were limit fed high concentrate or fed high roughage diets compared to cattle given ab libitum access to the high concentrate diet. Limit fed steers expressed the highest efficiency during finishing of all three treatments, and cattle consuming high concentrate diet ad libitum had the lowest efficiencies likely as a result of their lower empty body weight gain. Overall empty body weight gain and efficiency was lowest for cattle consuming the high roughage diet; these results were likely consequence of lower empty body weight gains during the growing phase and elevated DMI throughout the study (Sainz et al., 1995).

Overall, backgrounding cattle, regardless of strategy, generally improves ADG during the finishing phase over cattle given ad libitum access to a high concentrate ration. Variation in regard to feedlot performance during finishing and carcass characteristics as a result of backgrounding strategies needs to be investigated further. McCurdy et al. (2010)

mentioned lower performance for wheat pasture cattle may be the result of high animal stress from transportation at a later stage in life compared with animals that enter a drylot backgrounding system post-weaning. Additionally, energy requirements for maintenance may be lower for cattle limit-fed high concentrate diets allowing for more energy to be diverted to growth during finishing (Sainz et al., 1995).

Effect of implants

Usage of growth promoting implants in the United States is common. A review by the National Animal Health Monitoring Survey (NAHMS, 2013) revealed that feedlots with greater than 8,000 hd implant steers and heifers at least 91.7% and 94.1% of the time respectively. Implanting steers increased ADG, feed conversion efficiency, and hot carcass weight (HCW) by 18%, 6%, and 5%, respectively (Bucket et al., 1996). A more pronounced response was reported by Presten (1999) with growth rate and feed conversion efficiency improved by 30% and 15%. According to Duckett et al. (1996) implanting steers increased ADG 18.0%, DMI 6.0%, feed efficiency 8.0%, HCW 5.0%, and REA 4.0% relative to steers not receiving implants; marbling scores and percentage grade choice were not impacted by implant usage. Similar feedlot performace was reported by Duckett at al. (2014), however, marbling scores reviewed by Kerth et al. (1996) and Ducket and Pratt (2014) were found to be lower for implanted cattle. Rush et al. (1989) investigated varying implant potencies during backgrounding and found no differences in marbling. Brandt et al. (1995) investigated two background strategies grazing pastures of similar quality at different intensities (71 d intensive grazing or 145 d season long) and their interactions with cattle receiving either no implant or 20 mg of estradiol and 200 mg of progesterone (SynovexS) on d 1 of grazing. No measurable differences during backgrounding were

contributed to implant strategy, DMI during the feedlot phase was higher for steers implanted with SynovexS, but no differences in any carcass traits resulted from implant usage. No differences were reported in marbling when Pritchard (2000) compared carcasses of cattle receiving a low potency implant early in the finishing phase and their nonimplanted contemporaries. From these results authors made inference that differences in marbling could be related to the timing as well as potency of the implant (Pritchard, 2000). Bruns et al. (2005) researched stage of growth and implant exposure by delivering no implant or 24 mg estradiol and 120 mg trenbolone acetate (Revalor S) administered either early (d 1) or d 57 of the finishing phase. Early implanted cattle had carcasses with marbling scores significantly lower than nonimplanted animals, and marbling in carcasses from cattle receiving the delayed implant were no different from control. Marbling scores and quality grades were similar for carcasses from steers fed either 1.06 NE_g or 1.19 NE_g diets during backgrounding prior to finishing (Loken et al., 2009) In that study all steers received 36 mg of zeranol (Ralgro) d 1 of backgrounding and were re-implanted on d 50 of finishing with 24 mg estradiol and 120 mg trenbolone acetate (Revalor S). Cox-O'Neil et al. (2017) implanted all steers with 40 mg estradiol and 200 mg trenbolone actetate (Revalor XS) and randomly assigned them to one of three background treatments: silage-based diet in drylot, grazing cover crops, or grazing corn residues supplemented with distiller grains. Backgrounding ADG was highest for cattle fed in the drylot, intermediate for cover crop, and lowest for corn residue. Higher marbling scores and greater percent choice were also reported for carcasses from cattle backgrounded in a dry lot and fed a silage-based diet when compared to cattle grazing crop residue or cover crops. Given implant payout was similar for all steers regardless of backgrounding treatment, differences

in ADG carcass characteristics are consequence lower metabolizable energy intake (MEI). Pritchard (2016) fed a single diet at either ad libitum or restricted levels limiting growth to < 1.82 kg/d for an initial 63 d. Cattle were assigned one of two implant strategies designed to provide equal payout of 28 mg estradiol and 200 mg trenbolone acetate. Synovex plus (28 mg estradiol and 200 mg trenbolone acetate) steers were implanted on d1 while Synovex Choice (14 mg estradiol and 100 mg trenbolone acetate) steers were implanted on day 1 and again on d 63 delivering equal implant payout while altering implant potency to coincide with restriction of energy intake. Similar marbling scores were obtained for ab libitum fed cattle regardless of implant strategy. Restricting energy intake decreased marbling, but delaying the second yielded a marbling score similar to cattle fed ab libitum regardless of implant. Pritchard (2016) concluded altering implant strategy and dietary energy management can be utilized to procure ideal ADG, efficiency, and quality grade. However, further research is needed to examine altering implant potency and plane of nutrition before finishing, and its impact on growth, feedlot performance, and carcass traits.

Summary

Dietary roughage concentrations have long been the focal point of research concerning ruminants. Manipulation of the R:C ratio can be utilized to meet the specific goals of an operation. Greater concentrations of roughage in a diet increases DMI while diluting energy content, often resulting in lower ADG. Backgrounding is a period between weaning and finishing that is designed to minimize deposition of adipose, by utilizing greater amounts of roughage to limit energy intake, As a result increases in carcass size with minimal impact on carcass quality are commonly observed. Ruminal fermentation is also impacted by roughage concentrations in the diet. Feeding a high concentrate diet

lowers rumen pH, alters fermentation products, and impacts various enzymatic reactions. Biohydrogenation works to degrade PUFA to resulting in increased amounts of CLA and SFA to flow from the rumen. When rumen pH is decreased by the inclusion of cereal grains lipolysis, the first step of biohydrogenation, is inhibited, thereby increasing rumen passage of PUFA to potentially be deposited in meat. Both backgrounding and limiting biohydrogenation are examples of manipulating roughage concentrations, and further research is needed to investigate how these changes interact with various technologies and their impact on feedlot performance and carcass characteristics.

Figure 1. 1 Daily DMI intake versus initial BW for steers. Adopted from Hicks et al. (1990)

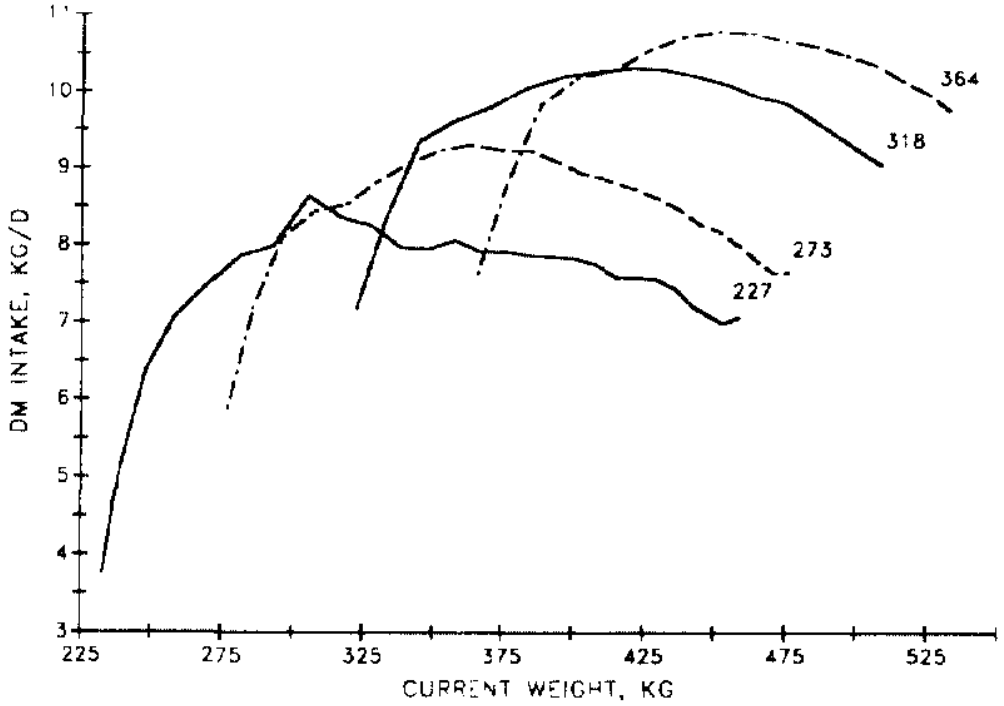


Figure 1. 2 Diagram of major pathways of ruminal fermentation. Adopted from NASEM (2016).

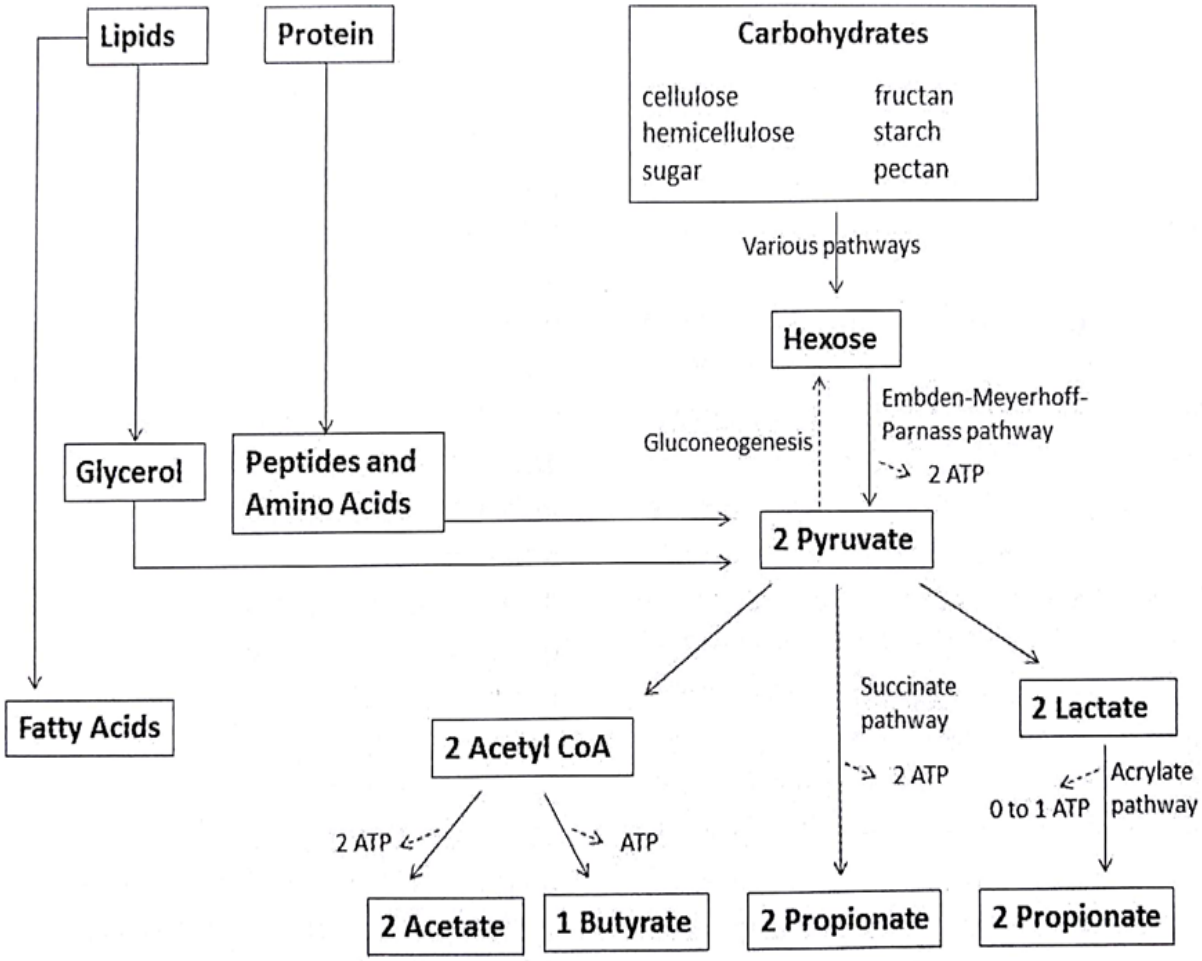


Figure 1. 3 Major pathways for the biohydrogenation of major PUFA. Adopted from Jenkins et al. (2008).

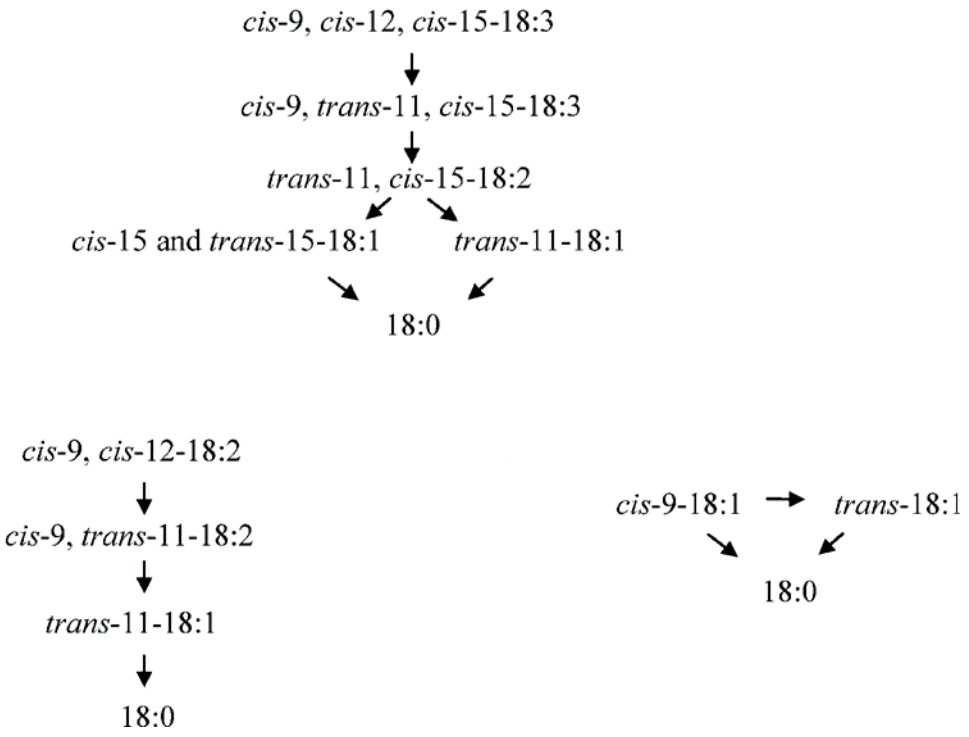
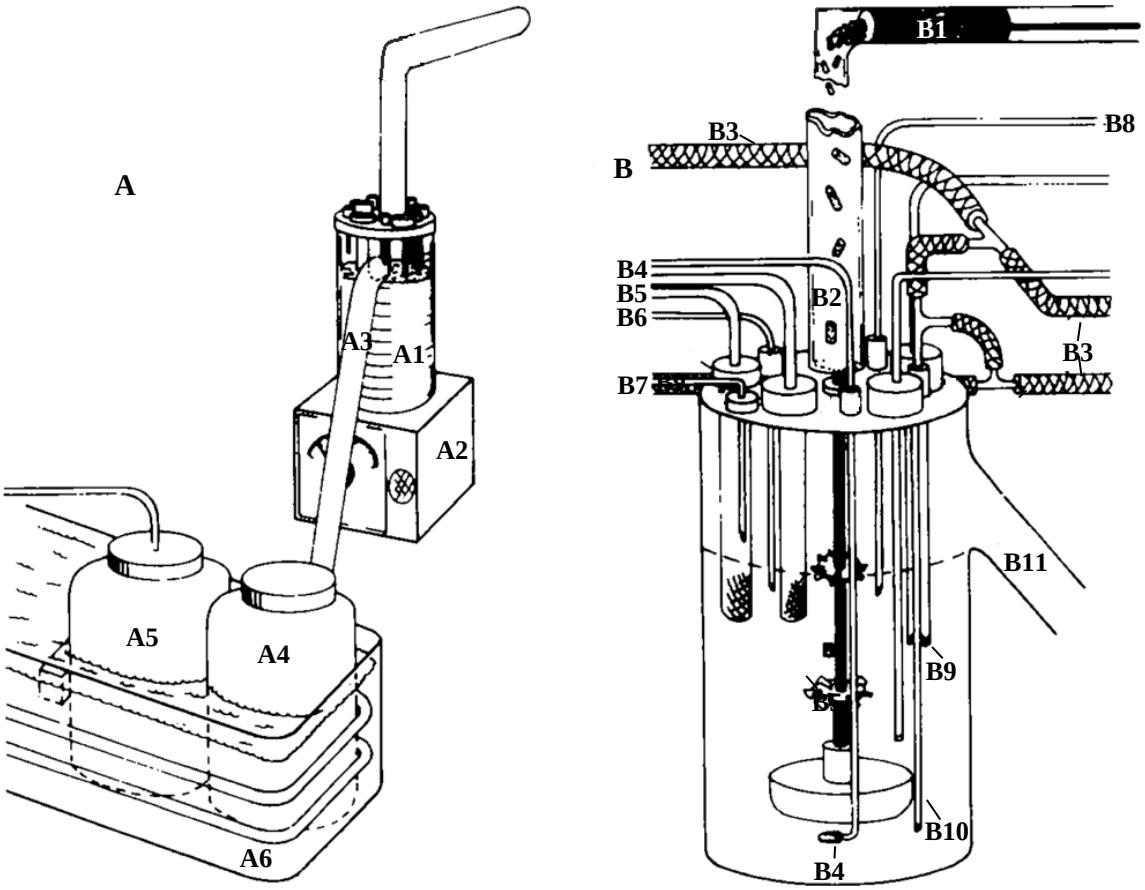


Figure 1. 4 General design of dual flow continuous culture system. Modified from Hannah et al. (1986) and Salfer (2015).



(A) General schematic of dual flow continuous culture fermenter system. **(A1)** Continuous culture fermentation vessel. **(A2)** Magnetic stir plate with temperature control. **(A3)** Solid effluent overflow tube. **(A4)** Solid effluent collection vessel. **(A5)** Liquid effluent collection vessel. **(A6)** Refrigerated water bath (2°C). **(B)** Fermentation vessel assembly. **(B1)** Feed delivery tube. **(B2)** Feed input port. **(B3)** Coaxial heat exchange apparatus. **(B4)** Nitrogen sparager. **(B5)** Liquid effluent filters. **(B6)** Hydrochloric acid input port. **(B7)** Buffer infusion port. **(B8)** Sodium hydroxide input port. **(B9)** pH electrode. **(B10)** Temperature probe. **(B11)** Solid effluent port.

Effects of feeding a moderate energy diet to coincide with initial release of 80 mg trenbolone acetate and 16 mg estradiol in a long-acting implant (Revalor-XS) on feedlot performance carcass characteristics

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Overview

It was hypothesized that the release of initial 80 mg trenbolone acetate and 16 mg estradiol from Revalor-XS implant would complement effects of feeding a moderate energy, growing diet for a short period without negative impacts on carcass quality. One-hundred ninety crossbred steers (353 ± 30 kg) were utilized in a generalized randomized block design experiment to determine the effect of a short-term, moderate-energy feeding phase in steers implanted with Revalor-XS on performance and carcass traits. Treatments consisted of feeding a moderate-energy diet (MdE; 1.26 NEg Mcal/kg for 63 d (within Revalor-IS delivery window) before transitioning to a high-energy diet during finishing or feeding a high-energy throughout the feeding period (HiE; 1.41 NEg Mcal/kg). Steers were initially blocked by weight and randomly allocated to 30 pens. Pens (n = 15) were randomly allocated to treatments. This resulted in heavier initial body weight (BW) ($P < 0.05$) for MdE steers (356 vs 349 kg). Subsequently, initial BW was used as a covariate instead of weight block. Steers were marketed when they were considered to have > 1.14 cm of fat cover as appraised visually. Days on feed (DOF) during finishing (113 d) or total days on feed (176 d) did not differ ($P > 0.10$). As expected, daily dry mater intake (DMI) and total DMI during the first 63 d were greater ($P < 0.01$) for steers fed MdE. A trend for greater DMI ($P < 0.10$) was observed during finishing for steers fed MdE. These differences led to steers fed MdE to have greater DMI ($P < 0.01$) during the entire feeding period. However, these differences were not reflected by total DMI during finishing or the entire feeding period. Average daily gain and feed conversion derived from live final BW or carcass-adjusted final BW were not affected ($P > 0.10$) by treatment. Because ADG was not affected by MdE, interim BW or final BW (live or carcass-adjusted) was not

different between treatments ($P > 0.10$). Carcasses of steers fed MdE had lower marbling scores ($P < 0.05$). This led to a trend for greater incidence of Select ($P = 0.09$) and lower incidence of premium Choice ($P = 0.02$) carcasses. Feeding a moderate-energy diet for 63 d before transitioning to a finishing diet in steers implanted with Revalor-XS led to similar feedlot performance albeit with lower reliance on concentrate feed ingredients. However, this advantage may be offset by reductions in carcass quality.

Key words: anabolic implants, energy intake, carcass characteristics

Introduction

Feeding cattle moderate- or low energy diets in a drylot (backgrounding or growing phase) for a period time prior to feeding a high-energy finishing diet increased DMI and improved ADG during finishing (Lancaster et al., 2014). Overall impact of backgrounding diets on carcass weight or quality is dependent on duration of backgrounding period and ADG during this phase (Johnson and DiCostanzo, 2017) and length of time during finishing (Lawrence and Pearce, 1964). When cattle are finished to a similar degree of finish, cattle fed a growing diet reached greater carcass weights while achieving similar carcass quality (Klopfenstein et al., 1999; Loken et al., 2009; McCurdy et al., 2010)

Growth-promoting implants are utilized by feedlot operators to increase performance and feed conversion efficiency (Preston, 1999). Use of this technology led to greater DMI which resulted in greater ADG and feed conversion efficiency (Duckett et al., 1996). Carcass weights, and ribeye area were reportedly greater when cattle received at least one implant. Overall, use of growth-promoting implants increased net return by $163/hd (Duckett and Pratt, 2014). When timed inappropriately, implanting cattle may reduce marbling (Duckett and Pratt, 2014) and increase skeletal maturity (Roeber et al., 2000). Use of appropriate hormone dose at the appropriate time (Bruns et al., 2005) at pre-harvest leads to little or no effect on marbling score. Using a sequentially-delivered implant containing 40 mg estradiol and 200 mg trenbolone acetate, Nichols et al (2014) observed no negative effects on marbling score.

Delaying implant administration until d 57 of finishing resulted in carcasses with similar marbling scores as their non-implanted cohorts (Bruns et al., 2005). Pritchard (2016) reported no difference for marbling scores between ad-libitum-fed cattle and cattle

whose energy intake was restricted when implant potency was reduced during the initial growing period.

Revalor-XS contains 40 mg estradiol and 200 mg trenbolone acetate in 10 total pellets; 6 of the pellets (24 mg estradiol and 120 mg trenbolone acetate) are coated in a proprietary polymer that delays degradation and mimics reimplantation. Therefore, we hypothesized that cattle fed a moderate-energy, growing diet prior to finishing would complement effects of low initial implant potency delivered by Revalor-XS and result in heavier carcasses without negative effects on carcass quality. Our objectives were to study effects of feeding a moderate-energy diet during the growing phase to coincide with initial low implant potency on feedlot performance and carcass characteristics.

Materials and methods

This experiment was conducted at the Beef Research and Educational Complex, Rosemount MN. All animal use protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Cattle and dietary treatments

One hundred ninety Angus x Simmental steers (353 ± 30 kg) were initially weighed and assigned to either light or heavy blocks. On d 1, following 7 d adaptation, steers received a growth-promoting implant containing 40 mg estradiol and 200 mg trenbolone acetate (Revalor-XS, Merck Animal Health), 2 mL of a 5-way respiratory vaccine (Pyramid 5, Boehringer Ingelheim, Ingelheim am Rhein, Germany), 5 ml of a 8-way clostridial vaccine (Covexin 8, Merck Animal Health, Madison, NJ), and 2 ml of a hoof rot vaccine (Fusogard, Elanco Animal Health, Greenfield, IN) . Within weight block, cattle were randomly assigned to pens (n = 30) in a 30-pen bedded barn (15 pens in a northern

and 15 pens in a southern row). Pens measured 3 m (width) by 10 m (length) and were stocked with 6 or 7 hd/pen (from 30 to 42 cm linear bunk space/hd or 4.3 to 5.0 m/hd). Water was accessible at all times through individual heated water tanks.

Pens were randomly assigned to one of two treatments consisting of feeding a moderate- (MdE) or high-energy (HiE) diet (Table 1). This resulted in 15 pens per treatment. Cattle in MdE were fed this diet for 63 d, after which they were transitioned in 7 d to consume HiE until the end of the experiment. Feeding took place at approximately 0800 h daily, and bunk scores and orts were collected daily at 0700 h. Orts were weighed and frozen until further analysis. Feed ingredient samples were taken weekly and frozen for further analysis. Corn silage and high-moisture corn were removed from bunker silos using a mechanical rake and transferred to a feed center every 2 d. Feeds were mixed in a vertical mixer (Patz, location) using the following order: corn silage, dry distillers grains, dry rolled corn, liquid supplement, and high-moisture corn; mixing started when dry ingredients were added and continued 5 min after last ingredient was added. Dietary (as-fed) concentrations of feed ingredients loaded daily were adjusted according to DM measurements of corn silage and high-moisture corn made when these feeds were removed from the silos.

During the growing phase, initial and final weight were obtained after withdrawing feed and water for 16 h. Interim weights were taken every 28 d before the morning feeding. Cattle were marketed when subjectively appraised to have greater than 1.14 cm of fat cover over the dorsal area, resulting in two separate shipping dates. Cattle were harvested at Iowa Premium Beef (Tama, IA), where carcass weight (HCW), loin muscle area (LMA), fat thickness (BF), Marbling, quality grade (QG), and yield grade (YG) as assessed by a USDA

grader were collected. Final BW was determined by dividing HCW by the common dressing percentage for the loads.

Chemical analyses

Feed and ort samples were analyzed for DM, NDF, EE, and CP. Dry-matter was determined by drying samples at 60^{o} C for 24 h in a forced-air oven (Blue M Electric, East Troy Wisconsin). Neutral-detergent fiber was analyzed utilizing an Ankom 200 Fiber Analyzer (ANKOM Technology Corp., Macedon, NY). Fat content was determined by extracting ether from samples with an Ankom XT10 Extraction System (ANKOM Technology Corp., Macedon, NY). Crude protein was calculated from estimating N content of feed samples using a Kjeltec 2300 Analyzer Unit following digestion at 410^{o} C for 1 h utilizing a Foss Digestor 2020 (Foss Tecator AB, Höganäs, Sweden).

Statistical analyses

Continuous performance data such as DMI, ADG, feed conversion efficiency, HCW, BF, marbling score (500 = small), YG, and LMA area were analyzed using Proc Mixed of SAS (SAS Institute, Cary, NC) as a randomized complete block design with initial BW as a covariate.

$$Y_{ijk} = \mu + t_i + \beta_j + \text{In } BW_{ij} + e_{ijk}$$

Where μ is the overall treatment mean, t_i represents treatment effect, β_j is block effect, In BW_{ij} is the covariate effect of initial BW, and e_{ijk} is the residual error. Initial BW covariate was deleted from the model if $P > 0.10$. Using this model, feed conversion efficiency was analyzed with ADG as Y_{ijk} except for DMI was also used as a covariate. Resulting least square means represent ADG for each treatment at the same DMI; a measure of feed

conversion efficiency. A measure of concentrate (mostly corn grain and corn co-products) feeding was generated by subtracting contribution of corn silage from total DMI. This value represents grain and grain co-products for which various livestock production systems compete. Carcass quality grade was analyzed utilizing Proc GLIMMIX of SAS (SAS Institute, Cary, NC). Significance was reported at $P < 0.05$ and trends were discussed at $P < 0.10$.

Results and Discussion

Feedlot performance

At the end of the growing period BW was similar ($P = 0.91$). Feeding a moderate energy diet resulted in an increase ($P < 0.01$) in DMI during the growing phase (Table 2.2). Despite an increase in DMI, no differences were found for ADG ($P = 0.98$) or G:F ($P = 0.5$). Greater dietary roughage concentration generally leads to greater DMI (Defoor and Galyean, 2003). Greater DMI observed in cattle consuming diets containing greater than 10% roughage (NDF% from Defoor and Galyean, 2003) may be due to greater gut size resulting from the stimulating effect of roughage in the rumen (Varga and Harpster, 1995). In contrast, feeding high concentrate diets may trigger satiety mechanisms that include chemostatic receptors (NRC, 1987). These factors may have contributed to greater DMI by cattle fed MdE in the current experiment. However, greater dietary roughage concentration is generally associated with lower ADG (Defoor et al., 2002). This was not observed in the current experiment. Greater than expected ADG by cattle fed MdE likely resulted from greater DMI and therefore similar metabolizable energy intake (MEI; $P = 0.55$). Yet, greater DMI by cattle fed MdE was not accompanied by a sufficient ADG to maintain feed conversion efficiency similar to that of cattle fed HiE (Table 2.2).

During the finishing phase, cattle fed MdE tended to consume more DM ($P = 0.09$); yet, greater DMI by cattle fed MdE resulted in no differences ($P = 0.18$) in ADG or feed conversion efficiency ($P = 0.24$). However, greater DMI did not result in greater MEI ($P > 0.05$), this observation could explain lack of differences between treatments. End weight or days on feed were not affected by energy content of the growing diet (Table 2.2). McCurdy et al. (2010) backgrounded cattle utilizing either wheat pasture, corn silage-based diets, or programmed intake of a high energy diet, with a control of cattle consuming a high energy diet ad-libitum. Finishing phase DMI was greater for all three background treatments over the control cattle who consumed an energy-dense ration for the duration of the trial. The wheat pasture and silage-based backgrounding diets also had greater finishing phase DMI than the programmed intake diet.McCurdy et al. (2010) also reported MEI during finishing was highest for silage-fed steers over control steers, and this was evident by increased ADG for steers consuming the silage-based diet. Lack of ADG differences in the current trial were likely result of similar MEI during finishing.

Overall feedlot performance is reported in Table 2.3. Across both phases, steers fed MdE had greater ($P < 0.01$) DMI; however, total DM consumption was not affected ($P = 0.45$) by growing diet energy content. Although statistically insignificant, DOF were greater for HiE cattle, likely explaining the lack of differences in total DM consumption. No differences in overall ADG, feed conversion efficiency or days on feed ($P = 0.24$) were observed between treatments. Hicks et al. (1990) compiled DMI data from commercial feedlots and reported that yearling cattle received into the feedlot after backgrounding had significantly greater DM than cattle entering the feedlot shortly after weaning; it was concluded this observation was result of increase in rumen volume developed while

consuming bulkier forages. Lack of performance differences in the current experiment was likely due to similar start weight at initiation of finishing and lack of any period of compensatory gain following feeding of a moderate diet. Greater than expected ADG for MdE cattle during the growing phase may have also contributed to lack of performance differences. Lancaster et al. (2014) reported that diets containing high and medium concentrations of starch consumed by growing cattle resulted in no differences in ADG and G:F during both backgrounding and finishing phases. Given our main source of roughage came from silage, which is commonly viewed as 50% roughage and 50% grain, starch content may have been elevated for MdE. Additionally, lack of performance differences during both low and high implant phases could be explained by similar MEI across phases.

Carcass characteristics

Carcass data are listed in Table 2.4. No differences were found between treatments for HCW ($P = 0.33$), BF ($P = 0.73$), and LMA($P = 0.68$). Yield grade was not ($P = 0.54$) affected by treatment. Carcasses of cattle fed HiE had greater ($P < 0.05$) marbling scores. A greater proportion of carcasses from cattle fed HiE graded upper 2/3 choice and prime ($P = 0.09$). In contrast, a greater proportion ($P = 0.02$) of carcasses from cattle fed MdE graded USDA Select. Treatment had no ($P = 0.72$) effect on proportion of carcasses grading lower 1/3 choice.

Carcass weights and loin muscle area, a proxy measure of muscling, were not different for cattle placed in a growing program (Lancaster et al., 2014). Additionally, Lancaster et al. (2014) reported no differences in YG for carcasses of both calf-fed and yearling cattle. Sainz et al. (1995) harvested steers directly after a period of growth

restriction to determine effects ofon carcass characteristics. Cattle consuming less energy, as observed in backgrounding diets, produced lower marbling scores (Sainz et al., 1995). Furthermore, cattle that consumed forage-based diets instead of limit-fed high-energy diets produced carcasses with lower marbling scores (Sainz et al., 1995). This demonstrates that substrate may affect adipose tissue deposition intramuscularly even when cattle are fed moderate-energy diets. However, this effect is not irreversible. Sainz et al. (1995) demonstrated that if cattle are given adequate time during finishing and are harvested at a constant back fat depth, suitable for industry standards, marbling not differ regardless of pre-finish treatment. Contrary to our results, McCurdy et al. (2010) reported greater marbling scores for cattle fed a silage-based diet during the growing phase in contrast to cattle consuming a high-energy diet ad-libitum. Dry matter intake by steers fed the silage-based diet by McCurdy et al. (2010) was increased resulting in a subsequent increase of MEI. Increased MEI by McCurdy et al. (2010) could be an explanation for the greater marbling by steers fed the silage-based diet. In the current trial no differences in MEI were observed and given that performance was similar for both treatments while carcass quality suffered, substrate may explain differences in marbling scores and quality grades.

Conclusion

Our results suggest feeding a moderate-energy diet to coincide with initial release of Revalor-XS increased DMI throughout the feeding period. However, ADG and feed efficiency were not affected by growing phase diet. Final adjusted BW did not differ between treatments, and both groups reached similar degrees of finish at similar DOF. No differences were detected for HCW, BF, and LMA; however, marbling score was reduced when a moderate-energy diet was fed during the growing phase. Given MEI was similar

for both treatments and no differences in performance traits were detected, different substrates provided during initial growing phase may account for differences reported for marbling. Further research is needed investigating effects of various substrates during the growing phase on carcass quality.

Table 2. 1 Diet composition during growing phase

	Treatment[1]	
Item	MdE	HiE
Ingredient, % DM basis		
Corn silage	38.3	9.9
High moisture corn	17.4	59.0
Dried distillers grains w/solubles	20.5	13.2
Dry rolled corn	18.2	14.0
Liquid supplement*	5.7	3.9
Nutrient composition		
DM, %	52.1	64.5
NDF, %	22.3	13.2
Fat, %	4.1	3.9
CP, %	15.3	13.9
NEg, Mcal/kg	1.33	1.41

[1]MdE = moderate-energy diet fed for initial 63 d; HiE = high-energy diet fed during initial 63 d and finishing phase.

*Contains Rumensin-90 at 400 g/ton

Table 2. 2 Effect of dietary energy on feedlot performance during growing and finishing phase

	Treatment[1]			
Item	MdE	HiE	SE	*P*-value
Growing phase				
In BW, kg	356	350	1.54	< 0.01
DMI, kg/d	6.74	6.45	0.29	< 0.01
MEI[2], Mcal/kg	24.08	23.90	0.21	0.55
ADG, kg	1.70	1.70	0.03	0.98
Gain to feed, kg	1.75	1.69		0.55
End BW, kg	460	460	1.62	0.96
Finishing phase				
DMI, kg/d	9.88	9.67	0.03	0.09
MEI[2], Mcal/kg	30.55	30.13	0.22	0.20
ADG, kg	1.38	1.32	0.03	0.18
Gain to feed, kg	2.74	2.80		0.24
End BW[3], kg	615	610	4.47	0.45
Total BW Gain, kg	155	150	4.15	0.43
Days on feed	112	114	2.27	0.68

[1]MdE = moderate energy diet fed for initial 63 d; HiE = high energy diet fed during initial 63 d and finishing phase.

[2]MEI = metabolizable energy intake

[3]Carcass-adjusted from dressing percent

Table 2. 3 Effect of dietary energy on total feedlot performance

	Treatment[1]			
Item	MdE	HiE	SE	*P*-value
DMI, kg/d	8.98	8.83	0.10	< 0.01
ADG, kg	1.49	1.45	0.03	0.24
Gain to feed, kg	0.17	0.16		0.74
Days on feed	175	177	2	0.68
Total DM, kg/hd	1,521	1,516	23	0.84
Total BW Gain, kg	261	256	4	0.45

[1]MdE = moderate energy diet fed for initial 63 d; HiE = high energy diet fed during initial 63 d and finishing phase.

Table 2. 4 Effects of dietary energy on carcass characteristics

Item	Treatment[1] MdE	HiE	SE	*P*-value
HCW, kg	390	387	0.98	0.33
Fat depth, cm	1.63	1.63	0.01	0.73
LMA, sq cm	85.99	85.61	0.07	0.68
Marbling[2]	440	468	8.83	0.03
YG	3.04	2.99	0.07	0.57

	Treatment[1] MdE		HiE	
	n	%	*n*	%
Choice, upper 2/3	16[a]	17.2	25[b]	26.9
Choice, lower 1/3	55	59.1	52	55.9
Select	22[b]	23.7	16[a]	17.2

[1] MdE = moderate energy diet fed for initial 63 d; HiE = high energy diet fed during initial 63 d and finishing phase.

[2] 300 = Slight, 400 = Small, 500 Modest.

[a,b] Means within row with different superscripts tended ($0.05 < P\text{-value} < 0.10$) to be different.

Effects of dietary-induced pH changes on feed digestion, nitrogen metabolism, fermentation, and biohydrogenation of polyunsaturated fatty acids in dual-flow continuous culture fermenters.

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Overview

Ruminal pH decreases as greater concentrations of grain are consumed. As a result, rumen function may be altered changing nutrient flow post-ruminally. Polyunsaturated fatty acids (PUFA) are extensively hydrogenated by rumen microbes to stearic acid 18:0, which is a saturated fatty acid (SFA). Our objective was to investigate effects of pH resulting from feeding grain or grass-based diets *in vitro* on biohydrogenation by ruminal microbes. Eight dual-flow, continuous culture fermenters (1,045 mL) were randomly assigned to 1 of 4 dietary treatments in a completely randomized block design. Dietary treatments consisted of feeding 100:0 roughage-to-concentrate ratio (Grass), 50:50 roughage-to-concentrate plus flax oil supplement (Mix+F), 10:90 roughage-to-concentrate ratio plus corn oil supplement (Con+C), or 10:90 roughage-to-concentrate ratio plus flax oil supplement (Con+F). Differences in fermenter pH were expected due to dietary composition; therefore, fermenter pH was regulated between 5.5 and 7.0. Liquid and solid flow rates were maintained at 8.66% and 4.51%, respectively, and fluid temperature was maintained at 38.5^{o} C. Average fermenter pH, area under the curve, and maximum pH were lowest for Con+C and Con+F ($P < 0.05$). Apparent and true DM, OM, and NDF digestibility were greater with grain inclusion ($P < 0.05$) while ADF digestibility was not affected by grain inclusion ($P > 0.05$). Crude protein digestibility was lower as grain inclusion increased ($P < 0.05$). Total VFA concentrations were lowest for Grass, highest for Con+C and Con+F, and intermediate for Mix+F ($P < 0.05$). Acetate-to-propionate ratio was lowest for Con treatments ($P < 0.05$). Biohydrogenation of both 18:2n-6 and 18:3n-3 fatty acids were reduced ($P < 0.01$) with the inclusion of concentrate. Stearic acid (18:0) recovered from lyophilized effluent was greatest for Grass, intermediate for Mix+F, and

least for Con+C and Con+F ($P < 0.05$). Linoleic acid (cis9,cis12-18:2) proportions in effluent were greatest for Con+C and Con+F and intermediate for Mix+F ($P < 0.05$). Between Con+C and Con+F concentrations of alpha-linolenic acid (cis9,cis12,cis15-18:3; ALA) was greater when flax oil was supplemented ($P < 0.01$). Proportions of ALA in effluent was similar ($P > 0.10$) between Grass and Con+C; however, between Mix+F and Con+F ALA concentrations tended ($P = 0.06$) to be greater for Con+F. Omega-6 to omega-3 ratios (n-6:n-3) were similar across Grass, Mix+F, and Con+F ($P > 0.10$) and greatest for Con+C ($P < 0.05$). Between high concentrate treatments, when corn oil was supplemented n-6:n-3 was significantly greater ($P < 0.01$) than when flax was incorporated into the diet. Observations of roughage-to-concentrate ratios effects on fermenter pH, nutrient digestibility, and nitrogen metabolism were expected and are consistent with those made in previous studies. The observed decrease in biohydrogenation activity in diets containing greater amounts of concentrate, accompanied by the impact of differing oil sources between high concentrate diets, indicate that diet type and oil supplement can be utilized to manipulate post-ruminal fatty acid profile.

Introduction

Diet composition during cattle feedlot finishing affects beef quality (Maughan et al., 2012) and beef nutrient composition (Muir et al., 1998; Wood et al., 2004). Recently, consumers' interest in type and concentration of fatty acids in meat has increased (Daley, 2010). Typically, fatty acid (FA) profile of fresh grass is comprised of approximately 50% to 75% linolenic acid (Hawke, 1973). In contrast, cereal grains contain greater concentrations of linoleic acid (Larson, 2016). Daley (2010) indicated that dietitians recommend FA ratio concentrations of omega-6 to omega-3 ratio (n6:n3) of 4:1; yet profile of FA in current American diets is estimated to be from 15:1 to 20:1 (Simopoulos, 2006). Response to greater interest in increasing the n6:n3 ratio in human diets, particularly by enhancing concentration of omega-3 FA alpha linolenic acid (ALA) in human diets, led to greater consideration of grass-finishing programs (Leheska et al., 2008).

Various supplemental FA sources and/or dietary strategies have been considered to enhance the concentration of ALA in meat and milk products derived from ruminants. Silva et al. (2016) reported greater concentrations of ALA in effluent when supplementing alfalfa hay-based diets with either flax or chia seed in a dual-flow continuous culture system in comparison with effluent from fermenters receiving an alfalfa hay-based diet plus a fat supplement containing low amounts of PUFA. Feeding high concentrate diets to cattle leads to lower ruminal pH, which may lower rate of lipolysis, the first step in fatty acid biohydrogenation. This resulted in lower rates of PUFA hydrogenation in continuous culture fermenters (Van Nevel and Demeyer, 1995; Jenkins et al., 2008). In the experiments by Van Nevel and Demeyer (1995) and Jenkins et al. (2008) fermenter pH was tightly regulated thereby preventing normal pH fluctuations commonly observed *in vivo* post

feeding. Therefore, the objective of this trial was to investigate effects of fermenter pH resulting from feeding grain or grass-based diets *in vitro* on biohydrogenation. We hypothesized that feeding greater amounts of concentrate would reduce fermenter pH and subsequent biohydrogenation of PUFA. Additionally, it was expected that including flax oil instead of corn oil in high concentrate diets would increase the flow of alpha-linolenic acid.

Materials and Methods

All animal procedures and protocols for this trial were approved by the University of Minnesota Animal Care and Use committee.

Diets and treatments

Four diets (Table 3.1) were formulated to represent three roughage:concentrate ratios, grass fed (Grass), 50/50 plus flax (Mix+F), and high concentrate (90%) plus flax or corn oil (Con+F or Con+C respectively). Ingredients were dried at 60^{o} C in a forced-air oven (Blue M Electric, East Troy Wisconsin) and ground in a Wiley No. 4 laboratory mill (Arthur H. Thomas Co. Philadelphia, PA) to pass through a 2 mm screen. Diet DM was measured at 100^{o} C for 24 h (Thelco laboratory oven, Precision Scientific, Chicago, IL) the day prior to inoculation and d 7 prior to collection phase for both periods. Feed was delivered to fermenters by hand twice daily at 0800 and 2000 h (Table 1). Changes in rumen volume, as a result of forage inclusion, cannot be replicated by fermenters due to their sold structure. Subsequently, feeding amounts had to be adjusted to more accurately represent expected rumen volumes. Feed amounts were determined by regressing DMI on forage intake and corrected for rumen volume based on data collected by Larson et al.

(unpublished) from Holstein steers consuming similar diets to those represented in the current experiment. Diet samples were taken on d 7 and frozen for further analysis.

Collection of rumen fluid and inoculation

Three ruminally-cannulated Holstein steers were used as fluid donors. Steers were fed a diet containing (DM basis) 50% alfalfa haylage, 45% dry-rolled corn, and 5% liquid supplement for at least 2 wk prior to collection. Rumen fluid (10 L) was collected, transported to campus and strained through four layers of cheesecloth. After straining, fluid was pooled across source steer, homogenized, and poured equally into eight preheated fermenters (1,045 mL). Immediately following inoculation, 25 g of diet was delivered to each fermenter to provide immediate substrate for the microbes.

Continuous culture design

Eight dual-flow continuous culture fermenters designed and operated as explained by Hannah et al. (1986) were used in two consecutive 10-d periods with 7 d of adaptation and 3 d of sampling. Artificial saliva (pH = 8.13), as described by Weller and Pilgrim (1974), was constantly infused to provide a final concentration (g/L) of $NaHCO_3$, 5.0; Na_2HPO_4, 1.76; $KHCO_3$, 1.6; KCL, 0.6; $MgSO_4$, 0.05; and urea, 0.4. Regulation of saliva input allowed for a liquid dilution rate of 8.66%/h, while solids dilution rates were maintained at 4.51%/h. Each fermenter pH was continuously monitored by an electronic data acquisition system (DASYLab v5.04, Measurement Computing, Norton, MA) and recorded every 5 sec. Fermenter pH was maintained between 5.5 and 7.0 with addition of either 5N sodium hydroxide (NaOH) or 3N hydrochloric acid (HCl). Addition of N_2 gas at a rate of 40 ml/min was used to maintain an anaerobic environment, and liquid temperature

was held constant at 38.5^{o} C using a coazial heat exchange apparatus. Fermenter contents were agitated constantly at 350 rpm utilizing a magnetic stir plate.

Sample collection

During the final 3 d or each period, solids and liquid effluents were maintained at 4^{o} C to limit microbial and enzymatic activity. For each fermenter, solids or liquid effluent were pooled each day of the sampling period and homogenized, after which 500 mL were collected and frozen for further analyses. A portion of each frozen sample was lyophilized (describe machine) after thawing at room temperature for 12 h, remaining effluent was then frozen again. Concentrations of DM, OM, NDF, ADF, FA, and purines were measured on the lyophilized sample. Remaining frozen effluent was thawed and subsampled for analysis of VFA, N, and NH_3-N. Following each 10-d experimental period, complete fermenter contents were filtered through 4 layers of cheesecloth and centrifuged at 1,000 x *g* to remove feed particles, and supernatant was subsequently centrifuged at 20,000 x *g* to separate microbial cells which were and analyzed for DM, OM, total N and purines.

Chemical analysis

Experimental diets, lyophilized effluent, and lyophilized microbial cells were dried in an oven at 100^{o} C for 24 h (Thelco laboratory oven, Precision Scientific, Chicago, IL) then combusted in a furnace (AOAC, 2005) at 550^{o} C for 12 h. Neutral detergent fiber and ADF was determined using an ANKOM 200 fiber analyzer (ANKOM Corp, Fairport, NY). Liquid effluent was centrifuged (5,000 x g) and supernatant was analyzed for NH_3-N with a Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). Total N was determined on diet, effluent, and lyophilized microbial samples utilizing a Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). Lyophilized effluent and microbial

cells were analyzed for purine concentrations according to Zinn and Owens (1986). Purine-to-N ratio was used to determine flow of bacterial N and OM in effluent samples. Effluent samples were shipped to the North Florida Research and Education Center (Marianna, FL) for determination of VFA concentrations following the procedure described by (Goetsch and Galyean, 1983). Fatty acid concentrations were determined for diet and effluent samples that were shipped to Penn State University (Happy Valley, PA) and analyzed as directed by Kielanowski (1976).

Statistical analysis

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC) as a randomized complete block design:

$$Y_{ijkl} = \mu + t_i + \beta_j + P_k + e_{ijkl}$$

Where μ represents overall mean, t_i equals effect of treatment, β_j random effect of block, P_k random effect of period, and e_{ijkl} as residual error. Average pH was analyzed using a similar model with h as a repeated measure. Amount of base used in each fermenter was used as a covariate to model effects of treatment on pH and area under the curve. This variable was retained in the model if $P < 0.05$.

Results and discussion

pH parameters

Fermenter pH results are displayed in Figure 3.1 and Table 3.2. Average fermenter pH was highest for fermenters fed Grass, lowest for Con+C and Con+F diets, and intermediate for Mix+F ($P < 0.05$). Minimum pH was highest for Grass ($P < 0.05$?) and similar for Mix+F, Con+C, and Con+F ($P > 0.05$); this was expected as a minimum pH threshold was set at 5.5 for all fermenters. However, area under the curve (AUC) was

lowest for Con+C and Con+F, intermediate for Mix+F treatment and highest for Grass ($P < 0.05$).

Amounts of acid and base infused to maintain pH within set range are reported in Table 3.2. Greater amounts of 3N HCl were used to decrease fermenter pH below the 7.0 threshold for Grass treatment ($P < 0.05$) while amounts of 3N HCl used to control pH by Mix+F, Con+C, and Con+F were similar. Theoretically, no acid should have been needed by treatments containing grain; acid volatilization from reservoirs likely contributed to this value. Similar amounts of 5N NaOH were pumped for Con+C and Con+F ($P > 0.05$) fed fermenters. Even though minimum pH was similar for Con+C, Con+F and Mix+F treatments, less total base was needed to maintain pH above 5.5 for Mix+F fermenters ($P < 0.05$). Base used in fermenters fed Grass to maintain pH above 5.5 was lowest and should have been negligible; values reported likely resulted from the need to supplement base lost to volatilization.

Previous continuous culture research comparing similar dietary treatments in which pH was allowed to fluctuate with such a wide range (5.5 - 7.0) is limited. In continuous culture, Loor et al. (2003) included ground corn at 0%, 16%, and 32% of dietary DM in diets differing by roughage type (Orchardgrass or Red clover) and harvest season (fall or spring). Fermenter pH linearly decreased as grain inclusion increased; however, average fermenter pH never dropped below 6.75. *In vivo* results from Poor et al. (1990) indicated that minimum rumen pH was impacted by concentrate levels in the diet; steers were fed 30%, 60%, and 90% concentrate diets, and minimum rumen pH was reported to be 6.34, 5.76, and 5.40 respectively.

Digestibility

Digestibility results are reported in Table 3.2. Similar trends were found for apparent and true DM digestibility. As concentrate inclusion increased, apparent total and OM digestibility increased ($P < 0.05$). Interestingly, OM digestibility was greater for Con+F than Con+C ($P < 0.05$); the only difference in formulation of these diets was source of fat potentially demonstrating the greater toxicity of omega-3 fatty acids on microbial activity. Normally, degree of unsaturation has varying effects on digestion, with greater inhibition observed with unsaturated fatty acids (Palmquist, 1988). Flaxseed oil is reported as having greater concentrations of unsaturated FA (NRC, 2012), however a reduction in digestibility was not observed in the current trial. Digestibility of NDF was lowest for fermenters fed the Mix+F diet but not in fermenters fed either high concentrate or 100% roughage diets ($P < 0.05$). Feeding high-concentrate diets resulted in no differences in NDF digestibility ($P > 0.05$). Interestingly, Con+C and Grass diets were found to not differ in digestibility; this may have resulted from differences in roughage sources, elevated pH above typical *in vivo* pH observations, and increased fermenter retention time over what is commonly observed in a rumen. Lower pH commonly decreases fiber digestibility through inhibition of cellulolytic activity (Van Soest, 1982). Roughage source of Mix+F diets was primarily from ryegrass silage; inclusion of grain and corresponding lower pH may have inhibited cellulolytic bacterial activity.

Acid detergent fiber digestibility was not affected by dietary treatments ($P > 0.05$). Including increments of grain into roughage-based diets improved DM and NDF digestibility in continuous culture (Loor et al., 2003); however, inclusion of grain in that trial never exceeded 35%. Similar digestibility results were reported for dairy cows

consuming 0 , 5 , or 10 kg of corn supplement (Reis and Combs, 2000). Greater DM and OM digestion occurred when cows consumed either 5 or 10 kg of supplement with no impact on NDF and ADF digestion. Poore et al. (1990) found increasing concentrate content (30, 60, and 90%) of the diet increased total tract digestibility while having no impact on total tract NDF digestion. Authors concluded that similarities in NDF digestion were result of NDF source in the diet. In high concentrate diets a large proportion of NDF comes from concentrate rather than roughage and concentrate NDF has a higher potential for digestion when high grain diets are fed (Van Soest, 1982). This potentially explains increased NDF digestibility values reported in Table 3.2 for Con+C and Con+F compared to Mix+F.

Nitrogen metabolism

Table 3.3 lists N metabolism results. Fermenter NH_3-N concentrations were highest for Grass treatments ($P < 0.05$) and lowest for diets containing concentrate. Flow of NH_3-N, determined from daily effluent volumes, followed a similar trend as NH_3-N concentrations with Grass having greatest NH_3-N flow Con treatments the lowest ($P < 0.05$). Non-ammonia N concentrations in collected effluent was not different between Mix+F, Con+C, and Con+F ($P > 0.05$) and lowest overall for Grass ($P < 0.05$). No differences between treatment means were found for microbial N flow ($P > 0.05$). Flow of dietary N was lowest for Grass ($P < 0.05$) and did not differ between diets containing concentrate ($P > 0.05$). These results reflect differences in CP degradability, which was highest for Grass ($P < 0.05$) and did not differ between Mix+F, Con+C, and Con+F treatments ($P > 0.05$). Efficiency of microbial protein synthesis (EMPS) was influenced by diet type. Efficiency of microbial protein synthesis for Grass treatment was greater ($P <$

0.05) than that of Con+C and Con+F diets; however, no differences were found between Grass and Mix+F or Mix+F and Con+C ($P > 0.05$).

Reducing fermenter pH in, regardless of diet, led to lower CP degradation (Cardozo et al., 2002). Substrate rather than pH can have an impact on either fermenter or rumen NH_3-N concentrations. Lana et al. (1998) fed diets containing 0%, 45%, and 90% concentrate and reported that decreases in NH_3-N concentrations only occurred when 100% roughage diets were fed and pH was reduced below 6.2; no differences were reported when diets containing 45% and 90% concentrate were fed. The relationship between EMPS and rumen pH is discussed at length in the meta-analysis by St. Pierre (2000). The authors concluded that no relationship between pH and EMPS was evident, but rather more closely related with OM digestion. Loor et al. (2003) reported that as grain concentrations increased EMPS decreased.

Volatile fatty acid concentrations

Table 3.4 lists dietary effects on fermenter VFA concentration. Total VFA concentrations were greater for Con+F and Con+C when compared with Grass ($P < 0.05$). Concentrations of VFA for Mix+F were also greater than Grass ($P < 0.05$) but not different than Con+C ($P > 0.10$). These results further confirm previous literature that including concentrate in diets will increase fermentation and ultimately VFA concentrations (Lana et al., 1998). As expected, propionate concentrations were greatest for treatments containing grain ($P < 0.05$) while acetate concentrations were lowest for Grass ($P < 0.05$). Acetate to propionate ratio was highest ($P < 0.05$) for Grass. Butyrate concentration followed similar tendencies with Mix+F, Con+C, and Con+F treatments producing a greater concentration than Grass ($P < 0.05$). Total VFA and individual VFA concentrations

are similar to those reported by Fuentes et al. (2009). It is well understood that greater fermentation is associated with higher concentrate diets, which results in greater concentrations of VFA and lower acetate-to-propionate ratios (NASEM, 2016).

Fatty acids

Effects of treatment on biohydrogenation are reported in Table 3.6. Feeding Mix+F, Con+C, and Con+F resulted in no differences in ($P > 0.05$) biohydrogenation of *cis*9-18:1 (oleic acid), while Grass inhibited biohydrogenation to the greatest extent ($P < 0.05$). Increasing dietary concentrate decreased hydrogenation of 18:2*n*-6 and 18:3*n*-3; both classes of PUFA were hydrogenated the most when Grass was fed ($P < 0.05$), while Con+C and Con+F resulted in the lowest biohydrogenation. The Mix+F diet decreased efficiency of biohydrogenation compared to Grass ($P < 0.05$), but not to the same degree as either high-concentrate treatment. Similar result for *cis*9-18:1 were reported by Loor et al. (2003) when fermenters were fed one of three levels of corn supplement (0, 8, or 16 g). Feeding 50 g without corn supplementation of either Orchardgrass or Red clover resulted in lowest biohydrogenation activity of oleic acid (Loor et al., 2003). Loor et al. (2004) altered dietary roughage and concentrate proportions to produce diets of 65:35 or 35:65 roughage to concentrate and fed them to lactating Holstein cows. Similar results were reported for biohydrogenation of 18:2*n*-6 and 18:3*n*-3 fatty acids, with greater concentrate inclusion inhibiting biohydrogenation activity. Fuentes et al. (2009) demonstrated that lower pH, rather than substrate, inhibited biohydrogenation of PUFA. Therefore, decreased biohydrogenation observed in the current trial is likely due to lower fermenter pH (Table 3.2) as a result of feeding smaller proportions of roughage in Mix+F, Con+C, and Con+F diets.

Individual FA concentrations as a percent of total FA recovered from effluent are shown in Table 3.7. Total FA recovered from effluent was greatest for Mix+F diets, intermediate for Con+C and Con+F diets, and lowest for Grass diets (P-value). This can likely be explained by dietary concentrations of FA found in Table 3.1. Concentrations of stearate (18:0), the most common product of biohydrogenation (Jenkins et al., 2008), was greatest ($P < 0.05$) in effluent collected from fermenters fed Grass diets; biohydrogenation was most active in Grass fermenters (Table 3.6) so this result is expected. Oleic acid concentrations were similar ($P > 0.05$) for Con+C and Con+F and both were found to be greater ($P < 0.05$) than Mix+F and Grass. Specific CLA with potential human health benefits can be synthesized from oleic acid (Jenkins et al., 2008), and increasing passage of oleic acid through the rumen may offer opportunities to improve current health benefits associated with meat and milk derived from ruminants. Linoleic acid (*cis*9,*cis*12-18:2), an omega-6 FA commonly known for negative human health impacts when consumed is excess (Leheska et al., 2008), was greater ($P < 0.05$) in Con+C and Con+F than ?. This result can be explained by Larson (2016) who stated cereal grains contain greater ($P < 0.05$) proportions of linoleic acid compared with the FA profile of different forage sources. Both Mix+F and Con+F diets increased ($P < 0.05$) concentrations of ALA (*cis*9,*cis*12,*cis*15-18:3) recovered from effluent compared with Grass and Con+F. Interestingly, no difference ($P > 0.05$) was detected between Grass and Con+F for ALA recovery. Given dietary concentrations of omega-3 FA (Table 3.1) were substantially greater for Grass, similar ALA concentrations found in effluent further demonstrate greater effects of biohydrogenation reported for Grass diets. Similar ($P > 0.05$) omega-6:omega-3 ratios were reported for Grass, Mix+F, and Con+F, and all three were greater ($P > 0.05$)

than Con+C. Dietitians recommend humans consume a diet that produces an omega-6:omega-3 ration no greater than 4:1, and finishing cattle on grass produces a ratio similar to the one desired by dietitians (Daley, 2010). These results provide evidence that feeding greater proportions of roughage and supplementing flax, a source of omega-3 FA, could provide substrate post-ruminally to be deposited in either meat or milk, further improving current health benefits associated with products derived from ruminants.

Conclusion

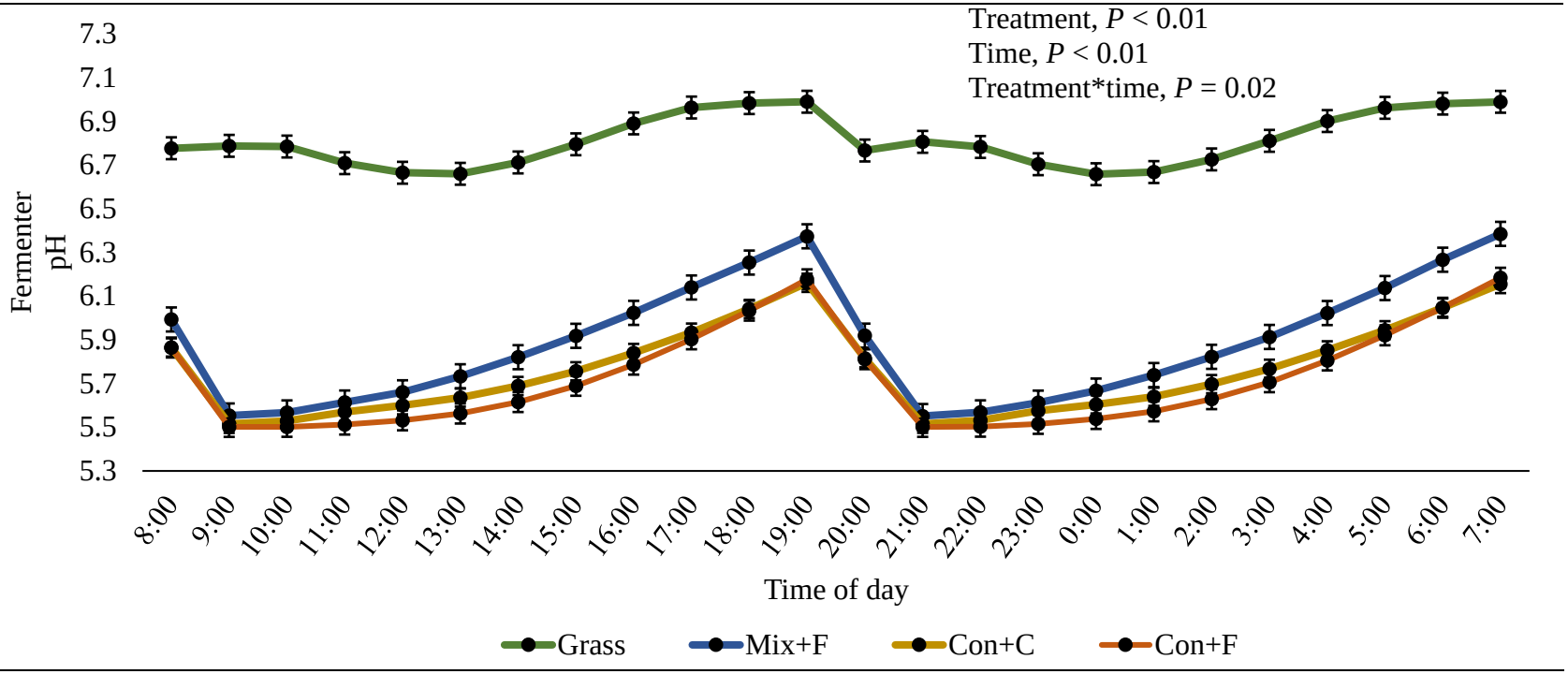
Results from this trial confirm that dietary concentrate concentrations play a critical role in altering fermenter pH parameters. As expected, apparent and true DM and OM digestibility's were greatest for high concentrate diets. While fiber digestion was also higher for concentrate diets, this may have resulted from varying NDF sources and respective fermenter pH. Fermentation, as measured by VFA concentration, was increased with concentrate inclusion, however, percent CP degradation and EMPS decreased in response to increases in dietary concentrate concentration.

Table 3. 1 Ingredients and composition

	Treatment[1]			
Item[2]	Grass[3]	Mix+F	Con+C	Con+F
Steam flaked corn	--	45.8	73.5	73.5
Ryegrass silage	--	38.0	--	--
Soybean meal	--	--	10.0	10.0
Soybean hulls	--	10.0	10.0	10.0
Liquid supplement	--	3.7	4.5	4.5
Corn oil	--	--	1.7	--
Flax oil	--	2.5	--	1.7
Chemical Compostion[2]				
DM	95.23	91.74	83.21	88.51
OM	88.89	97.97	86.95	92.17
CP	16.55	21.15	20.30	21.70
N	3.38	2.65	3.25	3.47
NDF	48.25	20.85	15.45	16.41
ADF	25.25	16.47	9.54	7.97
Total fatty acids	1.75	3.89	1.48	2.40
Fatty acid profile[4]				
16:0	21.78	11.72	33.14	24.86
18:0	22.96	3.81	5.92	5.25
c*is*9-18:1	3.61	21.61	27.89	34.14
18:2*n*-6	16.50	29.48	23.01	24.03
18:3*n*-3	49.20	28.92	2.31	4.96

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.
[2] Values reported on DM basis
[3] Composite of orchardgrass and reed canary
[4] Values reported as % of total FA

Figure 3. 1 Variation in fermenter pH over a 24-h period[1]



[1] Feeding took place at 8:00 and 20:00; pH data was collected every 5 s and averaged over 1 h starting at 8:00.

[2] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil

Table 3. 2 Effect of dietary treatment on pH parameters

	Treatment[1]				
Measurement	Grass	Mix+F	Con+C	Con+F	SEM
Average pH	6.81[a]	5.89[b]	5.76[c]	5.74[c]	0.03
Maximum	7.01[a]	6.56[b]	6.29[c]	6.32[c]	0.08
Minimum	6.53[a]	5.48[b]	5.46[b]	5.42[b]	0.05
AUC, 5.5 to 7.0	160[a]	136[b]	129[c]	129[c]	
HCl, mL/d	8.03[a]	0.40[b]	0.38[b]	0.42[b]	0.38
NaOH mL/d	0.25[a]	21.5[b]	53.3[c]	49.3[c]	3.91

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.

[a,b,c] Differing superscripts within row represent differences between treatment means ($P < 0.05$).

Table 3. 3 Effect of dietary treatment on nutrient digestibility

	Treatment[1]				
Digestion, %	Grass	Mix+F	Con+C	Con+F	SE
DM, apparent	32.8[a]	53.8[b]	57.3[c]	61.9[d]	5.43
DM, true	41.1[a]	56.1[b]	63.0[c]	65.1[c]	4.89
OM, apparent	39.1[a]	57.1[b,c]	61.6[c,d]	63.9[d]	0.99
OM, true	43.8[a]	60.7[b]	70.0[c]	69.0[c]	1.46
NDF	65.6[a,b]	62.3[a]	71.4[b,c]	76.0[c]	2.27
ADF	71.6	77.4	76.7	77.3	2.12

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.

[a,b,c,d] Differing superscripts within row represent differences between treatment means ($P < 0.05$).

Table 3. 4 Effect of dietary treatment on nitrogen metabolism

	Treatment[1]				
Item	Grass	Mix+F	Con+C	Con+F	SE
NH_3-N, mg/dL	4.81[a]	3.42[a,b]	2.47[b,c]	1.35[c,d]	0.78
N flow, g/d					
NH_3-N	0.11[a]	0.07[a,b]	0.05[b,c]	0.03[c]	0.02
Non NH_3-N	1.16[a]	1.71[b]	1.81[b]	1.67[b]	0.09
Microbial N	0.36	0.22	0.52	0.40	0.13
Dietary N	0.77[a]	1.31[b]	1.19[b]	1.12[b]	0.16
CP degradation, %	72.8[a]	48.1[b]	55.6[b]	56.0[b]	0.05
EMPS[2]	40.5[a]	34.9[a,b]	28.2[b,c]	22.4[c]	3.77

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.

[a,b,c] Differing superscripts within row represent differences between treatment means ($P < 0.05$).

[2]EMPS: efficiency of microbial protein synthesis (g of microbial N/kg of OM truly digested).

Table 3. 5 Effects of treatment on VFA production

	Treatment[1]				
Volatile fatty acids	Grass	Mix+F	Con+C	Con+F	SE
Total VFA, mM	89.2[a]	152[b]	176[b,c]	180[c]	9.88
Acetate	65.1[a]	80.7[b]	83.7[b]	92.5[b]	4.77
Propionate	16.9[a]	35.4[b]	44.9[b]	40.0[b]	8.98
Butyrate	5.48[a]	24.7[b]	32.2[b]	33.2[b]	5.00
Isobutyrate	0.41	0.36	0.39	0.30	0.06
Valerate	0.78[a]	10.6[b,c]	14.5[d]	13.6[c,d]	1.46
Acetate:propionate	3.85[a]	2.78[b,c]	1.94[d]	2.41[c,d]	0.57

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.

[a,b,c,d] Differing superscripts within row represent differences between treatment means ($P < 0.05$).

Table 3. 6 Effect of dietary treatment on biohydrogentation % of PUFA

	Treatment[1]				
Fatty acids[2], %	Grass	Mix+F	Con+C	Con+F	SE
c*is*9-18:1	40.85[a]	66.29[b]	65.42[b]	63.16[b]	5.68
18:2*n*-6	91.48[a]	81.22[b]	58.74[c]	59.53[c]	3.48
18:3*n*-3	95.36[a]	83.91[b]	62.06[c]	63.31[c]	4.45

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.

[2] Biohydrogenation = [(intake of fatty acid – overflow of fatty acid) / intake of fatty acid] x 100

[a,b,c] Differing superscripts within row represent differences between treatment means ($P < 0.05$).

Table 3. 7 Effect of dietary treatment on fatty acid concentrations of lyophilized effluent

	Treatment[1]				
Fatty acids[2], %	Grass	Mix+F	Con+C	Con+F	SE
Total, mg	616[a]	1606[b]	1286[c]	1029[c]	78.6
14:0	1.70[a]	0.67[b]	0.55[b]	0.71[b]	0.23
16:0	16.46[a]	11.02[b]	12.95[c]	11.34[b]	1.06
17:0	1.13[a]	0.41[b]	0.51[b]	0.51[b]	0.01
18:0	49.57[a]	11.89[b]	4.42[b]	3.90[b]	1.45
*cis*9-18:1	4.48[a]	11.99[b]	19.42[c]	19.56[c]	0.60
*trans*10-18:1	0.82[a]	24.83[b]	19.58[bc]	15.86[c]	1.23
*cis*11-18:1	2.17[a]	1.42[b]	1.28[b]	1.26[b]	0.18
*trans*11-18:1	6.70[a]	4.19[ab]	1.67[b]	2.36[b]	1.31
*cis*9,*cis*12-18:2	1.82[a]	8.97[b]	26.54[c]	24.87[c]	1.85
trans11,*cis*15-18:2	1.34[a]	7.22[b]	0.99[a]	4.55[c]	0.62
*cis*6,*cis*9,*cis*12-18:3	0.84	0.56	0.28	0.11	0.01
*cis*9,*cis*12,*cis*15-18:3	2.99[a]	7.53[b]	1.74[a]	10.04[b]	1.13
24:0	0.78[a]	0.36[b]	0.26[c]	0.22[b]	0.01
n-6:*n*-3	1.19[a]	1.29[a]	9.11[b]	2.73[a]	2.31

[1] Grass = grass fed; Mix+F = 50/50+flax; Con+C = high concentrate+corn oil; Con+F = high concentrate+flaxseed oil.

[2] Values reported as a % of total fatty acid concentrations

[a,b,c,] Differing superscripts within row represent differences between treatment means ($P < 0.05$).

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